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NEWS 30 Oct 24 Nutraceuticals International (NUTRACEUT) now available on  
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=> nucleocapsid

L1 8446 NUCLEOCAPSID

=> pseudo and L1

L2 12 PSEUDO AND L1

=> eukaryotic (w) virus

L3 148 EUKARYOTIC (W) VIRUS

=> L3 and L2

L4 0 L3 AND L2

=> flavivirus and L2

L5 0 FLAVIVIRUS AND L2

=> "hepatitis C virus"

L6 30819 "HEPATITIS C VIRUS"

=> L6 and L2

L7 2 L6 AND L2

=> virus and L2

L8 12 VIRUS AND L2

=> tRNA and L2

L9 0 TRNA AND L2

=> tRNA

L10 42320 TRNA

=> L1 and L10

L11 159 L1 AND L10

=> L11 and L2

L12 0 L11 AND L2

=> L6 and L11

L13 1 L6 AND L11

=> "large spheral viral like particle"

L14 0 "LARGE SPHERAL VIRAL LIKE PARTICLE"

=> "viral like particel"

L15 0 "VIRAL LIKE PARTICEL"

=> "viral like particle"

L16 54 "VIRAL LIKE PARTICLE"

=> L16 and pseudo

L17 1 L16 AND PSEUDO

=> D L17 IBIB TI SO AU ABS 1

L17 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1968:473932 CAPLUS

DOCUMENT NUMBER: 69:73932

TITLE: The self-assembly of a nucleic-acid free  
**pseudo-top** component for a small spherical  
virus

AUTHOR(S): Bancroft, J. B.; Wagner, G. W.; Bracker, C. E.

CORPORATE SOURCE: Purdue Univ., Lafayette, Indiana, USA

SOURCE: Virology (1968), 36(1), 146-9

CODEN: VIRLAX

DOCUMENT TYPE: Journal

LANGUAGE: English

TI The self-assembly of a nucleic-acid free **pseudo-top** component  
for a small spherical virus

SO Virology (1968), 36(1), 146-9

CODEN: VIRLAX

AU Bancroft, J. B.; Wagner, G. W.; Bracker, C. E.

AB Protein, isolated from cowpea chlorotic mottle virus, when dialyzed  
overnight at 6.degree. against 0.2M NaCl-0.01M NaOAc, pH 5.0, formed  
nucleic acid-free spheres, which unlike any naturally occuring plant  
virus  
top component, differed in charge from the virus. The particles were  
52S,  
had a dry diam. of 250 A., and an electrophoretic mobility in 0.2M NaCl  
of  
4.3 .times. 10<sup>-5</sup> cm.<sup>2</sup>v.<sup>-1</sup>sec.<sup>-1</sup>. The particles did not differ  
antigenically from the virus.

=> L16 and L11

L18 0 L16 AND L11

=> D L13 IBIB TI SO AU ABS 1

L13 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:357237 CAPLUS

DOCUMENT NUMBER: 137:89946

TITLE: Conformational Changes Accompanying Self-Assembly of  
the **Hepatitis C Virus**  
Core Protein

AUTHOR(S): Kunkel, Meghan; Watowich, Stanley J. *Inventor*  
CORPORATE SOURCE: Department of Human Biological Chemistry & Genetics  
and the Sealy Center for Strucutal Biology,



University of Texas Medical Branch, Galveston, TX,  
77555, USA

SOURCE: Virology (2002), 294(2), 239-245  
CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Elsevier Science

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Conformational Changes Accompanying Self-Assembly of the **Hepatitis C Virus** Core Protein

SO Virology (2002), 294(2), 239-245  
CODEN: VIRLAX; ISSN: 0042-6822

AU Kunkel, Meghan; Watowich, Stanley J.

AB Although a no. of recent studies have suggested that the function of the **hepatitis C virus** (HCV) core protein may be both to package the viral genome and to modulate host cellular processes, little is known of the structure of the core protein necessary to accomplish these functions. Using in vitro assembled particles that mimic essential features of native HCV **nucleocapsids**, we report the earliest structural information of the HCV core protein and its **nucleocapsid**. The core protein is proteinase-resistant when assembled into **nucleocapsid**-like particles or complexed with nucleic acid in vitro. In contrast, the highly basic amino terminus of the free core protein is sensitive to proteolytic digestion. The hydrophobic carboxyl-terminal region of the core protein stabilizes the structure of the free core protein but is not required to stabilize core protein assembled into **nucleocapsid**-like particles or complexed to nucleic acid. Significantly, the carboxyl-terminal region is sufficient, but not necessary, to fold the core protein into a stable structure. These data are consistent with a model of a partially flexible HCV core protein that undergoes extensive conformational changes upon binding to nucleic acid and assembling into **nucleocapsid** particles. In addn., the susceptibility of **nucleocapsid** particles to RNase digestion suggests that RNA-core interactions may stabilize HCV **nucleocapsids**.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE REFORMAT

=> D L7 IBIB TI SO AU ABS 1-2

L7 ANSWER 1 OF 2 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:56543 CAPLUS

DOCUMENT NUMBER: 126:101682

TITLE: Chimeric Sindbis viruses dependent on the NS3 protease of **hepatitis C virus**

AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni

CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, 00040, Italy

SOURCE: Journal of Virology (1997), 71(2), 1417-1427  
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Chimeric Sindbis viruses dependent on the NS3 protease of

**hepatitis C virus**

SO Journal of Virology (1997), 71(2), 1417-1427  
 CODEN: JOVIAM; ISSN: 0022-538X

AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni

AB The **hepatitis C virus** (HCV) NS3 protease  
 cleaves the viral polyprotein at specific sites to release the putative  
 components of the HCV replication machinery. Selective inhibition of  
 this enzyme is predicted to block virus replication, and NS3 is thus  
 considered an attractive candidate for development of anti-HCV therapeutics. To set  
 up a system for anal. of NS3 protease activity in cultured cells, we  
 constructed a family of chimeric Sindbis viruses which carry sequences  
 coding for NS3 and its activator, NS4A, in their genomes. HCV sequences  
 were fused to the gene coding for the Sindbis virus structural  
 polyprotein via an NS3-specific cleavage site, with the expectation that processing  
 of the chimeric polyprotein, **nucleocapsid** assembly, and generation  
 of viable viral particles would occur only upon NS3-dependent  
 proteolysis.

Indeed, the chimeric genomes encoding an active NS3 protease produced  
 infectious viruses in mammalian cells, while those encoding NS3  
 inactivated by alanine substitution of the catalytic serine did not.  
 However, in infected cells chimeric genomes recombined, splicing out HCV  
 sequences and reverting to **pseudo**-wild-type Sindbis virus. To  
 force retention of HCV sequences, we modified one of the initial chimeras  
 by introducing a second NS3 cleavage site in the Sindbis virus portion of  
 the recombinant polyprotein, anticipating that revertants not encoding an  
 active NS3 protease would not be viable. The resulting chimera produced  
 infectious viruses which replicated at a lower rate than the parental  
 construct and displayed a marked temp. dependence in the formation of  
 lysis plaques yet stably expressed NS3.

L7 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 1997:81269 BIOSIS  
 DOCUMENT NUMBER: PREV199799387972  
 TITLE: Chimeric Sindbis viruses dependent on the NS3 protease of  
**hepatitis C virus**.  
 AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)  
 CORPORATE SOURCE: (1) Istituto di Ricerche di Biologia Molecolare P.  
 Angeletti, Via Pontina km 30.600, 00040 Pomezia Italy  
 SOURCE: Journal of Virology, (1997) Vol. 71, No. 2, pp.  
 1417-1427.

ISSN: 0022-538X.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English

TI Chimeric Sindbis viruses dependent on the NS3 protease of  
**hepatitis C virus**.

SO Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.  
 ISSN: 0022-538X.

AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)

AB The **hepatitis C virus** (HCV) NS3 protease  
 cleaves the viral polyprotein at specific sites to release the putative  
 components of the HCV replication machinery. Selective inhibition of this  
 enzyme is predicted to block virus replication, and NS3 is thus  
 considered

an attractive candidate for development of anti-HCV therapeutics. To set  
 up a system for analysis of NS3 protease activity in cultured cells, we  
 constructed a family of chimeric Sindbis viruses which carry sequences

coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein

via an NS3-specific cleavage site, with the expectation that processing of

the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis.

Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temperature dependence in the formation of lysis plaques yet stably expressed NS3.

=> D L2 IBIB TI SO AU ABS 1-12

L2 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:652537 CAPLUS

TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**

AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel

CORPORATE SOURCE: Department of Genetics and Microbiology, University of

Geneva School of Medicine, Centre Medicale Universitaire, Geneva, CH1211, Switz.

SOURCE: RNA (2002), 8(8), 1056-1067  
CODEN: RNARFU; ISSN: 1355-8382

PUBLISHER: Cambridge University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**

SO RNA (2002), 8(8), 1056-1067  
CODEN: RNARFU; ISSN: 1355-8382

AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste;

Ruigrok, Rob W. H.; Kolakofsky, Daniel

AB The minus-strand genome of Sendai virus is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is assocd. with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats.

Paramyxoviruses

that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC),

whose

hexamer phase is conserved within each virus group. To det. whether the

hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chem. modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepd. a matched set of viruses in which a model editing site was displaced 1 nt at a time relative to the genome ends. The relative abilities of these Sendai viruses to edit their mRNAs in cell culture infections were examd.,

and the ability of DMS to chem. modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied.

Cytidines at hexamer phases 1 and 6 were the most accessible to chem. modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is assocd. with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS

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L2 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:351514 CAPLUS

DOCUMENT NUMBER: 137:90675

TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein

AUTHOR(S): Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.

CORPORATE SOURCE: Department of Clinical and Experimental Medicine, Division of Microbiology, St Orsola General Hospital, University of Bologna, Bologna, 40138, Italy

SOURCE: Journal of General Virology (2002), 83(5), 1005-1012  
CODEN: JGVIAY; ISSN: 0022-1317

PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein

SO Journal of General Virology (2002), 83(5), 1005-1012  
CODEN: JGVIAY; ISSN: 0022-1317

AU Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.

AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. Here, the expression and localization of the UL53 gene

product was analyzed. The results showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level.

Immunoelectron microscopy studies have disclosed that nuclear **pseudo**-inclusions are labeled, whereas **nucleocapsid** formations within the intranuclear skein are neg. Furthermore, the

mature virus particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homolog in herpes simplex virus type 1.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

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L2 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:56543 CAPLUS

DOCUMENT NUMBER: 126:101682

TITLE: Chimeric Sindbis viruses dependent on the NS3 protease

of hepatitis C virus

AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni

CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare P. Angeletti, Pomezia, 00040, Italy

SOURCE: Journal of Virology (1997), 71(2), 1417-1427

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Chimeric Sindbis viruses dependent on the NS3 protease of hepatitis C virus

SO Journal of Virology (1997), 71(2), 1417-1427

CODEN: JOVIAM; ISSN: 0022-538X

AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni

AB The hepatitis C virus (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block virus replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for anal. of

NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis viruses which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo-wild-type** Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a

second

NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temp. dependence in the formation of lysis plaques yet stably expressed NS3.

L2 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:626027 CAPLUS

DOCUMENT NUMBER: 125:267435

TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length

AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel

CORPORATE SOURCE: Dep. Genetics Microbiology, Univ. Geneva Sch. Med., Geneva, Switz.

SOURCE: RNA (1996), 2(10), 1033-1045

CODEN: RNARFU; ISSN: 1355-8382  
PUBLISHER: Cambridge University Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

TI Paramyxovirus RNA editing and the requirement for hexamer genome length  
SO RNA (1996), 2(10), 1033-1045  
CODEN: RNARFU; ISSN: 1355-8382

AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel  
AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the  
programmed insertion of G residues into a short G run contained within a  
larger purine run, via **pseudo**-templated transcription. The  
templates for paramyxovirus transcription are genome **nucleocapsids**  
in which each nucleoprotein subunit is assocd. with 6 nt, and only  
genomes  
whose lengths are multiples of 6 are found naturally or are replicated  
efficiently in transfected cell systems. We have examd. the effect of  
varying total genome length on the frequency and no. of insertions into  
the mRNA editing site in a transfected cell system, using constructs that  
generate mini-genome analogs. We found that, as long as the purine run  
sequence and the region immediately upstream were unaltered, editing  
occurred during mRNA synthesis independent of the precise length of the  
mini-genome. However, when mini-genome constructs whose lengths were not  
multiples of 6 were used, insertions (or deletions) occurred during  
antigenome synthesis within the purine run, which strikingly restored the  
hexamer length. Genome length correction due to changes in the  
antigenome  
purine run length occurred only when the mini-genome was not a multiple  
of  
6, and these changes were only poorly affected by mutations in the mRNA  
editing site and the region immediately upstream. Our results suggest  
that the mRNA editing site is a natural hotspot for viral polymerase  
slippage during genome replication, and that this site serves the dual  
and  
complementary function of maintaining hexamer genome length. The unusual  
requirement of paramyxoviruses for genomes of precise hexamer length may  
have evolved to maintain genome stability against insertions in the mRNA  
editing site during replication.

L2 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 1995:361797 CAPLUS  
DOCUMENT NUMBER: 122:209332  
TITLE: A **pseudo**-revertant of a Sindbis virus 6K  
protein mutant, which corrects for aberrant particle  
formation, contains two new mutations that map to the  
ectodomain of the E2 glycoprotein

AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton  
J.  
CORPORATE SOURCE: Dep. of Mol. Microbiology, Washington Univ. School of  
Medicine, St. Louis, MO, 63110-1073, USA

SOURCE: Virology (1995), 206(2), 1027-34  
CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic  
DOCUMENT TYPE: Journal  
LANGUAGE: English

TI A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which  
corrects for aberrant particle formation, contains two new mutations that  
map to the ectodomain of the E2 glycoprotein  
SO Virology (1995), 206(2), 1027-34  
CODEN: VIRLAX; ISSN: 0042-6822

AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.

AB Most site-directed mutations in the gene encoding the small, membrane-assocd. 6K protein of Sindbis virus interfere selectively with virus assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic anal. of this revertant showed that 2 addnl. mutations, which were mapped to the ectodomain of the E2 virus glycoprotein, were present in addn. to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal cells. Five new virus genetic constructs were prepd. by insertion of these mutations into the wild-type virus. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the virus. These results indicate that 6K may play an indirect role in the packing of the virus spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L2 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1991:507301 CAPLUS

DOCUMENT NUMBER: 115:107301

TITLE: Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses

AUTHOR(S): Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.

CORPORATE SOURCE: Sch. Med., Univ. South. California, Los Angeles, CA, 90033, USA

SOURCE: Virology (1991), 183(2), 647-57

CODEN: VIRLAX; ISSN: 0042-6822

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses

SO Virology (1991), 183(2), 647-57

CODEN: VIRLAX; ISSN: 0042-6822

AU Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.

AB The hemagglutinin-esterase (HE) membrane glycoprotein is present only in some members of the coronavirus family, including some strains of mouse hepatitis virus (MHV). In the JHM strain of MHV, expression of the HE gene is variable and corresponds to the no. of copies of a UCUAA pentanucleotide sequence present at the 3'-end of the leader RNA. This copy no. varies among MHV strains, depending on their passage history. The JHM isolates with 2 copies of UCUAA in their leader RNA showed a high level of HE expression, whereas the JHM isolate with 3 copies had a low-level expression. In this study, the anal. of HE gene expression was extended to other MHV strains. The synthesis of HE mRNA in these viruses also correlates with the copy no. of UCUAA in the leader RNA and the particular intergenic sequence preceding the HE gene. In 1 MHV strain, MHV-1, no detectable HE mRNA was synthesized, despite the presence of a proper transcription initiation signal. This lack of HE mRNA expression was consistent with a leader RNA contg. three UCUAA copies. However, mutations and deletions within the coding region of the MHV-1 HE gene

have generated a stretch of sequence which resembled the transcriptional initiation motif, and was shown to initiate the synthesis of a novel smaller mRNA. These findings strengthened the theory that interactions

between leader RNA and transcriptional initiation sequences regulate MHV subgenomic mRNA transcription. Sequence anal. revealed that most MHV strains, through extensive mutations, deletions, or insertions, have lost the complete HE open reading frame, thus turning HE into a pseudogene. This high degree of variation is unusual as the other three structural proteins (spike, membrane, and **nucleocapsid**) are well-maintained. In contrast to bovine coronavirus, which apparently requires HE for viral replication, the HE protein in MHV may be only an accessory protein which is not necessary for viral replication. JHM and MHV-S, however, have preserved the expression of HE protein.

L2 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 2002:472042 BIOSIS  
 DOCUMENT NUMBER: PREV200200472042  
 TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**.  
 AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)  
 CORPORATE SOURCE: (1) Department of Genetics and Microbiology, University of Geneva School of Medicine, CMU, 9 Ave de Champel, CH1211, Geneva; Daniel.Kolakofsky@Medecine.unige.ch Switzerland  
 SOURCE: RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067. <http://uk.cambridge.org/journals/rna/>. print. ISSN: 1355-8382.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 TI Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai virus **nucleocapsids**.  
 SO RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067. <http://uk.cambridge.org/journals/rna/>. print. ISSN: 1355-8382.  
 AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)  
 AB The minus-strand genome of Sendai virus is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is associated with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats. Paramyxoviruses that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC), whose hexamer phase is conserved within each virus group. To determine whether the hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chemical modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepared a matched set of viruses in which a model editing site was displaced 1 nt at a time relative to the genome ends.  
 The relative abilities of these Sendai viruses to edit their mRNAs in cell culture infections were examined, and the ability of DMS to chemically modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied. Cytidines at hexamer phases 1 and 6 were the most accessible to chemical modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is



associated with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.

L2 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 2002:345323 BIOSIS  
DOCUMENT NUMBER: PREV200200345323  
TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.  
AUTHOR(S): Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.  
CORPORATE SOURCE: (1) Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, St Orsola General Hospital, Via Massarenti 9, 40138, Bologna: dalmonte@med.unibo.it Italy  
SOURCE: Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. <http://vir.sgmjournals.org>. print. ISSN: 0022-1317.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.  
SO Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. <http://vir.sgmjournals.org>. print. ISSN: 0022-1317.  
AU Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.  
AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. In this work, the expression and localization of the UL53 gene product was analysed. Results obtained showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level. Immunoelectron microscopy studies have disclosed that nuclear **pseudo-inclusions** are labelled, whereas **nucleocapsid** formations within the intranuclear skein are negative. Furthermore, the mature virus particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homologue in herpes simplex virus type 1.

L2 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 1997:81269 BIOSIS  
DOCUMENT NUMBER: PREV199799387972  
TITLE: Chimeric Sindbis viruses dependent on the NS3 protease of hepatitis C virus.  
AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)  
CORPORATE SOURCE: (1) Istituto di Ricerche di Biologia Molecolare P. Angeletti, Via Pontina km 30.600, 00040 Pomezia Italy  
SOURCE: Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427. ISSN: 0022-538X.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
TI Chimeric Sindbis viruses dependent on the NS3 protease of hepatitis C

virus.

SO Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.  
ISSN: 0022-538X.

AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)

AB The hepatitis C virus (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block virus replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for analysis of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis viruses which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious viruses in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious viruses which replicated at a lower rate than the parental construct and displayed a marked temperature dependence in the formation of lysis plaques yet stably expressed NS3.

L2 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1996:524119 BIOSIS

DOCUMENT NUMBER: PREV199699246475

TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length.

AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)

CORPORATE SOURCE: (1) Dep. Genet. Microbiol., Univ. Geneva Sch. Med., CMU, 9 Ave de Champel, CH 1211 Geneva Switzerland

SOURCE: RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045.  
ISSN: 1355-8382.

DOCUMENT TYPE: Article

LANGUAGE: English

TI Paramyxovirus RNA editing and the requirement for hexamer genome length.

SO RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045.  
ISSN: 1355-8382.

AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)

AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is associated with 6 nt, and only genomes whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examined the effect of varying total genome length on the frequency and number of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogues. We found that, as long as the purine run sequence and the region immediately upstream were

unaltered, editing occurred during mRNA synthesis independent of the precise length of the minigenome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions)

occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the antigenome purine run length occurred only when the mini-genome was not a multiple of 6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase slippage during genome replication, and that this site serves the dual and complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L2 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1995:156639 BIOSIS

DOCUMENT NUMBER: PREV199598170939

TITLE: A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein.

AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)

CORPORATE SOURCE: (1) Dep. Mol. Microbiol., Box 8230, Washington Univ. Sch. Med., St. Louis, MO 63110-1073 USA

SOURCE: Virology, (1995) Vol. 206, No. 2, pp. 1027-1034.

ISSN: 0042-6822.

DOCUMENT TYPE: Article

LANGUAGE: English

TI A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein.

SO Virology, (1995) Vol. 206, No. 2, pp. 1027-1034.

ISSN: 0042-6822.

AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)

AB Most site-directed mutations in the gene encoding the small, membrane-associated 6K protein of Sindbis virus interfere selectively with

virus assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic analysis of this revertant showed that two additional mutations, which were mapped to the ectodomain of the E2 virus glycoprotein, were present in addition to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal calls. Five new virus genetic constructs were prepared by insertion of these mutations into the wild-type virus. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the virus. These results indicate that 6K may play an indirect role in the packing of the virus spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L2 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1993:327067 BIOSIS

DOCUMENT NUMBER: PREV199396035417  
 TITLE: Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV).  
 AUTHOR(S): Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)  
 CORPORATE SOURCE: (1) Dep. Biol., P.O. Box 4010, Georgia State Univ., Atlanta, GA 30302 USA  
 SOURCE: Virology, (1993) Vol. 194, No. 2, pp. 585-596. ISSN: 0042-6822.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 TI Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating virus (LDV).  
 SO Virology, (1993) Vol. 194, No. 2, pp. 585-596. ISSN: 0042-6822.  
 AU Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)  
 AB The apparently complete sequence of the RNA genome of the neurovirulent isolate of lactate dehydrogenase-elevating virus (LDV-C) has been determined. The LDV-C genome is at least 14,222 nucleotides in length and contains eight open reading frames (ORFs). ORF 1a, which encodes a protein of 242.8 kDa and is located at the 5' end of the genome, contains at least two putative papain-like cysteine protease domains, and one putative chymotrypsin-like serine protease domain. This ORF terminates with a UAG stop codon that can be bypassed if a -1 frameshift occurs. The frameshift region consists of a heptanucleotide "slippery" sequence, 5'-UUUAAAC-3', followed by a putative **pseudo-knot**. ORF 1b encodes a protein of 155.4 kDa containing, in its N-terminal portion, an RNA-dependent RNA polymerase and an RNA helicase domain separated by a Zn finger domain. Another domain of unknown function that is also conserved in coronaviruses and toroviruses is located at the C-terminus of the ORF 1b product. Three cleavage sites in the ORF 1a polyprotein and three in the ORF 1b polyprotein were predicted for the chymotrypsin-like protease and tentatively delimit the mature nonstructural proteins of LDV. Six small, overlapping 3' ORFs (ORFs 2 through 7) encode proteins with calculated sizes of 25.8, 21.6, 19.8, 23.9, 18.9, and 12.3 kDa. ORF 7 encodes the virion **nucleocapsid** protein Vp-1, while ORF 6 encodes the nonglycosylated envelope protein Vp2. ORFs 5, 4, 3, and 2 each encode glycoproteins which may be virion envelope proteins. LDV is closely related to equine arteritis virus, Lelystad virus (LV), and simian hemorrhagic fever virus. These four viruses belong to a new group of positive-strand RNA viruses and are related to coronaviruses and toroviruses.

=> D L8 IBIB TI SO AU ABS 1-12

L8 ANSWER 1 OF 12 CAPLUS COPYRIGHT 2002 ACS  
 ACCESSION NUMBER: 2002:652537 CAPLUS  
 TITLE: Chemical modification of nucleotide bases and mRNA editing depend on hexamer or nucleoprotein phase in Sendai **virus nucleocapsids**  
 AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq, Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel  
 CORPORATE SOURCE: Department of Genetics and Microbiology, University of

Geneva School of Medicine, Centre Medicale  
Universitaire, Geneva, CH1211, Switz.

SOURCE: RNA (2002), 8(8), 1056-1067  
CODEN: RNARFU; ISSN: 1355-8382

PUBLISHER: Cambridge University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Chemical modification of nucleotide bases and mRNA editing depend on  
hexamer or nucleoprotein phase in Sendai **virus**  
**nucleocapsids**

SO RNA (2002), 8(8), 1056-1067  
CODEN: RNARFU; ISSN: 1355-8382

AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq,  
Jean-Baptiste;  
Ruigrok, Rob W. H.; Kolakofsky, Daniel

AB The minus-strand genome of Sendai **virus** is an assembly of the  
**nucleocapsid** protein (N) and RNA, in which each N subunit is  
assocd. with precisely 6 nt. Only genomes that are a multiple of 6 nt  
long replicate efficiently or are found naturally, and their replication  
promoters contain sequence elements with hexamer repeats.

Paramyxoviruses  
that are governed by this hexamer rule also edit their P gene mRNA during  
its synthesis, by G insertions, via a controlled form of viral RNA  
polymerase "stuttering" (**pseudo**-templated transcription). This  
stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC),  
whose  
hexamer phase is conserved within each **virus** group. To det.  
whether the hexamer phase of a given nucleotide sequence within  
**nucleocapsids** affected its sensitivity to chem. modification, and  
whether hexamer phase of the mRNA editing site was important for the  
editing process, we prepd. a matched set of **viruses** in which a  
model editing site was displaced 1 nt at a time relative to the genome  
ends. The relative abilities of these Sendai **viruses** to edit  
their mRNAs in cell culture infections were examd., and the ability of  
DMS  
to chem. modify the nucleotides of this cis-acting signal within resting  
viral **nucleocapsids** was also studied. Cytidines at hexamer  
phases 1 and 6 were the most accessible to chem. modification, whereas  
mRNA editing was most extensive when the stutter-site C was in positions  
2  
to 5. Apparently, the N subunit imprints the nucleotide sequence it is  
assocd. with, and affects both the initiation of viral RNA synthesis and  
mRNA editing. The N-subunit assembly thus appears to superimpose another  
code upon the genetic code.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR  
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RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L8 ANSWER 2 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:351514 CAPLUS

DOCUMENT NUMBER: 137:90675

TITLE: Analysis of intracellular and intraviral localization  
of the human cytomegalovirus UL53 protein

AUTHOR(S): Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N.  
M.; Perret, E.; Prevost, M. C.; Landini, M. P.

CORPORATE SOURCE: Department of Clinical and Experimental Medicine,  
Division of Microbiology, St Orsola General Hospital,  
University of Bologna, Bologna, 40138, Italy

SOURCE: Journal of General Virology (2002), 83(5), 1005-1012

CODEN: JGVIAY; ISSN: 0022-1317  
PUBLISHER: Society for General Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

TI Analysis of intracellular and intraviral localization of the human  
cytomegalovirus UL53 protein  
SO Journal of General Virology (2002), 83(5), 1005-1012  
CODEN: JGVIAY; ISSN: 0022-1317  
AU Dal Monte, P.; Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.;  
Prevost, M. C.; Landini, M. P.  
AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved  
herpesvirus genes. Here, the expression and localization of the UL53  
gene  
product was analyzed. The results showed that pUL53 is a new structural  
protein. In infected human fibroblasts, pUL53 localizes in cytoplasmic  
perinuclear granular formations together with other structural viral  
proteins. In the nucleus, pUL53 forms patches at the nuclear periphery  
and co-localizes with lamin B at the internal nuclear membrane level.  
Immunoelectron microscopy studies have disclosed that nuclear  
**pseudo**-inclusions are labeled, whereas **nucleocapsid**  
formations within the intranuclear skein are neg. Furthermore, the  
mature  
**virus** particle maintains pUL53 at its tegumental level. These  
data suggest that pUL53 could be involved either in **nucleocapsid**  
maturation or in the egress of **nucleocapsids** from the nucleus to  
the cytoplasm through the nuclear membrane, a role compatible with the  
function hypothesized for UL31, its positional homolog in herpes simplex  
**virus** type 1.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR  
THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L8 ANSWER 3 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:56543 CAPLUS

DOCUMENT NUMBER: 126:101682

TITLE: Chimeric Sindbis **viruses** dependent on the  
NS3 protease of hepatitis C **virus**

AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio,  
Giovanni

CORPORATE SOURCE: Istituto di Ricerche di Biologia Molecolare P.  
Angeletti, Pomezia, 00040, Italy

SOURCE: Journal of Virology (1997), 71(2), 1417-1427  
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Chimeric Sindbis **viruses** dependent on the NS3 protease of  
hepatitis C **virus**

SO Journal of Virology (1997), 71(2), 1417-1427

CODEN: JOVIAM; ISSN: 0022-538X

AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni

AB The hepatitis C **virus** (HCV) NS3 protease cleaves the viral  
polyprotein at specific sites to release the putative components of the  
HCV replication machinery. Selective inhibition of this enzyme is  
predicted to block **virus** replication, and NS3 is thus considered  
an attractive candidate for development of anti-HCV therapeutics. To set  
up a system for anal. of NS3 protease activity in cultured cells, we  
constructed a family of chimeric Sindbis **viruses** which carry  
sequences coding for NS3 and its activator, NS4A, in their genomes. HCV

sequences were fused to the gene coding for the Sindbis virus structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious **viruses** in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences and reverting to **pseudo**-wild-type Sindbis virus. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis virus portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera produced infectious **viruses** which replicated at a lower rate than the parental construct and displayed a marked temp. dependence in the formation of lysis plaques yet stably expressed NS3.

L8 ANSWER 4 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:626027 CAPLUS

DOCUMENT NUMBER: 125:267435

TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length

AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel

CORPORATE SOURCE: Dep. Genetics Microbiology, Univ. Geneva Sch. Med., Geneva, Switz.

SOURCE: RNA (1996), 2(10), 1033-1045  
CODEN: RNARFU; ISSN: 1355-8382

PUBLISHER: Cambridge University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

TI Paramyxovirus RNA editing and the requirement for hexamer genome length  
SO RNA (1996), 2(10), 1033-1045  
CODEN: RNARFU; ISSN: 1355-8382

AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel

AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is assocd. with 6 nt, and only genomes

whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examd. the effect of varying total genome length on the frequency and no. of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogs. We found that, as long as the purine run sequence and the region immediately upstream were unaltered, editing occurred during mRNA synthesis independent of the precise length of the mini-genome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions) occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the

antigenome

purine run length occurred only when the mini-genome was not a multiple of

6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase

and slippage during genome replication, and that this site serves the dual complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L8 ANSWER 5 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:361797 CAPLUS

DOCUMENT NUMBER: 122:209332

TITLE: A **pseudo**-revertant of a Sindbis **virus** 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein

AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.

CORPORATE SOURCE: Dep. of Mol. Microbiology, Washington Univ. School of Medicine, St. Louis, MO, 63110-1073, USA

SOURCE: Virology (1995), 206(2), 1027-34

CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic

DOCUMENT TYPE: Journal

LANGUAGE: English

TI A **pseudo**-revertant of a Sindbis **virus** 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein

SO Virology (1995), 206(2), 1027-34

CODEN: VIRLAX; ISSN: 0042-6822

AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton J.

AB Most site-directed mutations in the gene encoding the small, membrane-assocd. 6K protein of Sindbis **virus** interfere selectively with **virus** assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic anal. of this revertant showed that 2 addnl. mutations, which were mapped to the ectodomain of the E2 **virus** glycoprotein, were present in addn. to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal cells. Five new **virus** genetic constructs were prepd. by insertion of these mutations into the wild-type **virus**. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the **virus**. These results indicate that 6K may play an indirect role in the packing of the **virus** spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L8 ANSWER 6 OF 12 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1991:507301 CAPLUS

DOCUMENT NUMBER: 115:107301

TITLE: Heterogeneity of gene expression of the hemagglutinin-esterase (HE) protein of murine coronaviruses

AUTHOR(S): Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.

CORPORATE SOURCE: Sch. Med., Univ. South. California, Los Angeles, CA,



90033, USA  
 SOURCE: Virology (1991), 183(2), 647-57  
 CODEN: VIRLAX; ISSN: 0042-6822  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 TI Heterogeneity of gene expression of the hemagglutinin-esterase (HE)  
 protein of murine coronaviruses  
 SO Virology (1991), 183(2), 647-57  
 CODEN: VIRLAX; ISSN: 0042-6822  
 AU Yokomori, Kyoko; Banner, Lisa R.; Lai, Michael M. C.  
 AB The hemagglutinin-esterase (HE) membrane glycoprotein is present only in  
 some members of the coronavirus family, including some strains of mouse  
 hepatitis **virus** (MHV). In the JHM strain of MHV, expression of  
 the HE gene is variable and corresponds to the no. of copies of a UCUAA  
 pentanucleotide sequence present at the 3'-end of the leader RNA. This  
 copy no. varies among MHV strains, depending on their passage history.  
 The JHM isolates with 2 copies of UCUAA in their leader RNA showed a high  
 level of HE expression, whereas the JHM isolate with 3 copies had a  
 low-level expression. In this study, the anal. of HE gene expression was  
 extended to other MHV strains. The synthesis of HE mRNA in these  
**viruses** also correlates with the copy no. of UCUAA in the leader  
 RNA and the particular intergenic sequence preceding the HE gene. In 1  
 MHV strain, MHV-1, no detectable HE mRNA was synthesized, despite the  
 presence of a proper transcription initiation signal. This lack of HE  
 mRNA expression was consistent with a leader RNA contg. three UCUAA  
 copies. However, mutations and deletions within the coding region of the  
 MHV-1 HE gene have generated a stretch of sequence which resembled the  
 transcriptional initiation motif, and was shown to initiate the synthesis  
 of a novel smaller mRNA. These findings strengthened the theory that  
 interactions between leader RNA and transcriptional initiation sequences  
 regulate MHV subgenomic mRNA transcription. Sequence anal. revealed that  
 most MHV strains, through extensive mutations, deletions, or insertions,  
 have lost the complete HE open reading frame, thus turning HE into a  
 pseudogene. This high degree of variation is unusual as the other three  
 structural proteins (spike, membrane, and **nucleocapsid**) are  
 well-maintained. In contrast to bovine coronavirus, which apparently  
 requires HE for viral replication, the HE protein in MHV may be only an  
 accessory protein which is not necessary for viral replication. JHM and  
 MHV-S, however, have preserved the expression of HE protein.

L8 ANSWER 7 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 2002:472042 BIOSIS  
 DOCUMENT NUMBER: PREV200200472042  
 TITLE: Chemical modification of nucleotide bases and mRNA editing  
 depend on hexamer or nucleoprotein phase in Sendai  
**virus nucleocapsids**.  
 AUTHOR(S): Iseni, Frederic; Baudin, Florence; Garcin, Dominique;  
 Marq,  
 Jean-Baptiste; Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)  
 CORPORATE SOURCE: (1) Department of Genetics and Microbiology, University of  
 Geneva School of Medicine, CMU, 9 Ave de Champel, CH1211,  
 Geneva: Daniel.Kolakofsky@Medecine.unige.ch Switzerland  
 SOURCE: RNA (New York), (August, 2002) Vol. 8, No. 8, pp.  
 1056-1067. <http://uk.cambridge.org/journals/rna/>. print.  
 ISSN: 1355-8382.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 TI Chemical modification of nucleotide bases and mRNA editing depend on  
 hexamer or nucleoprotein phase in Sendai **virus**  
**nucleocapsids**.

SO RNA (New York), (August, 2002) Vol. 8, No. 8, pp. 1056-1067.  
<http://uk.cambridge.org/journals/rna/>. print.  
ISSN: 1355-8382.

AU Iseni, Frederic; Baudin, Florence; Garcin, Dominique; Marq,  
Jean-Baptiste;

Ruigrok, Rob W. H.; Kolakofsky, Daniel (1)

AB The minus-strand genome of Sendai **virus** is an assembly of the **nucleocapsid** protein (N) and RNA, in which each N subunit is associated with precisely 6 nt. Only genomes that are a multiple of 6 nt long replicate efficiently or are found naturally, and their replication promoters contain sequence elements with hexamer repeats. Paramyxoviruses that are governed by this hexamer rule also edit their P gene mRNA during its synthesis, by G insertions, via a controlled form of viral RNA polymerase "stuttering" (**pseudo**-templated transcription). This stuttering is directed by a cis-acting sequence (3' UNN UUUUUU CCC),

whose

hexamer phase is conserved within each **virus** group. To determine whether the hexamer phase of a given nucleotide sequence within **nucleocapsids** affected its sensitivity to chemical modification, and whether hexamer phase of the mRNA editing site was important for the editing process, we prepared a matched set of **viruses** in which a model editing site was displaced 1 nt at a time relative to the genome ends. The relative abilities of these Sendai **viruses** to edit their mRNAs in cell culture infections were examined, and the ability of DMS to chemically modify the nucleotides of this cis-acting signal within resting viral **nucleocapsids** was also studied. Cytidines at hexamer phases 1 and 6 were the most accessible to chemical modification, whereas mRNA editing was most extensive when the stutter-site C was in positions 2 to 5. Apparently, the N subunit imprints the nucleotide sequence it is associated with, and affects both the initiation of viral RNA synthesis and mRNA editing. The N-subunit assembly thus appears to superimpose another code upon the genetic code.

L8 ANSWER 8 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 2002:345323 BIOSIS

DOCUMENT NUMBER: PREV200200345323

TITLE: Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.

AUTHOR(S): Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.

CORPORATE SOURCE: (1) Department of Clinical and Experimental Medicine, Division of Microbiology, University of Bologna, St Orsola General Hospital, Via Massarenti 9, 40138, Bologna: [dalmonte@med.unibo.it](mailto:dalmonte@med.unibo.it) Italy

SOURCE: Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. <http://vir.sgmjournals.org>. print.  
ISSN: 0022-1317.

DOCUMENT TYPE: Article

LANGUAGE: English

TI Analysis of intracellular and intraviral localization of the human cytomegalovirus UL53 protein.

SO Journal of General Virology, (May, 2002) Vol. 83, No. 5, pp. 1005-1012. <http://vir.sgmjournals.org>. print.  
ISSN: 0022-1317.

AU Dal Monte, P. (1); Pignatelli, S.; Zini, N.; Maraldi, N. M.; Perret, E.; Prevost, M. C.; Landini, M. P.

AB Human cytomegalovirus (HCMV) UL53 belongs to a family of conserved herpesvirus genes. In this work, the expression and localization of the UL53 gene product was analysed. Results obtained showed that pUL53 is a new structural protein. In infected human fibroblasts, pUL53 localizes in

cytoplasmic perinuclear granular formations together with other structural viral proteins. In the nucleus, pUL53 forms patches at the nuclear periphery and co-localizes with lamin B at the internal nuclear membrane level. Immunoelectron microscopy studies have disclosed that nuclear **pseudo**-inclusions are labelled, whereas **nucleocapsid** formations within the intranuclear skein are negative. Furthermore, the mature **virus** particle maintains pUL53 at its tegumental level. These data suggest that pUL53 could be involved either in **nucleocapsid** maturation or in the egress of **nucleocapsids** from the nucleus to the cytoplasm through the nuclear membrane, a role compatible with the function hypothesized for UL31, its positional homologue in herpes simplex **virus** type 1.

L8 ANSWER 9 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 1997:81269 BIOSIS  
 DOCUMENT NUMBER: PREV199799387972  
 TITLE: Chimeric Sindbis **viruses** dependent on the NS3 protease of hepatitis C **virus**.  
 AUTHOR(S): Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)  
 CORPORATE SOURCE: (1) Istituto di Ricerche di Biologia Molecolare P. Angeletti, Via Pontina km 30.600, 00040 Pomezia Italy  
 SOURCE: Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.

ISSN: 0022-538X.

DOCUMENT TYPE: Article

LANGUAGE: English

TI Chimeric Sindbis **viruses** dependent on the NS3 protease of hepatitis C **virus**.

SO Journal of Virology, (1997) Vol. 71, No. 2, pp. 1417-1427.  
 ISSN: 0022-538X.

AU Filocamo, Gessica; Pacini, Laura; Migliaccio, Giovanni (1)

AB The hepatitis C **virus** (HCV) NS3 protease cleaves the viral polyprotein at specific sites to release the putative components of the HCV replication machinery. Selective inhibition of this enzyme is predicted to block **virus** replication, and NS3 is thus considered an attractive candidate for development of anti-HCV therapeutics. To set up a system for analysis of NS3 protease activity in cultured cells, we constructed a family of chimeric Sindbis **viruses** which carry sequences coding for NS3 and its activator, NS4A, in their genomes. HCV sequences were fused to the gene coding for the Sindbis **virus** structural polyprotein via an NS3-specific cleavage site, with the expectation that processing of the chimeric polyprotein, **nucleocapsid** assembly, and generation of viable viral particles would occur only upon NS3-dependent proteolysis. Indeed, the chimeric genomes encoding an active NS3 protease produced infectious **viruses** in mammalian cells, while those encoding NS3 inactivated by alanine substitution of the catalytic serine did not. However, in infected cells chimeric genomes recombined, splicing out HCV sequences

and

reverting to **pseudo**-wild-type Sindbis **virus**. To force retention of HCV sequences, we modified one of the initial chimeras by introducing a second NS3 cleavage site in the Sindbis **virus** portion of the recombinant polyprotein, anticipating that revertants not encoding an active NS3 protease would not be viable. The resulting chimera

produced infectious **viruses** which replicated at a lower rate than the parental construct and displayed a marked temperature dependence in the formation of lysis plaques yet stably expressed NS3.

L8 ANSWER 10 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 1996:524119 BIOSIS  
 DOCUMENT NUMBER: PREV199699246475  
 TITLE: Paramyxovirus RNA editing and the requirement for hexamer genome length.  
 AUTHOR(S): Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)  
 CORPORATE SOURCE: (1) Dep. Genet. Microbiol., Univ. Geneva Sch. Med., CMU, 9 Ave de Champel, CH 1211 Geneva Switzerland  
 SOURCE: RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045. ISSN: 1355-8382.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 TI Paramyxovirus RNA editing and the requirement for hexamer genome length.  
 SO RNA (New York), (1996) Vol. 2, No. 10, pp. 1033-1045. ISSN: 1355-8382.  
 AU Hausmann, Stephane; Jacques, Jean-Philippe; Kolakofsky, Daniel (1)  
 AB Paramyxoviruses cotranscriptionally edit their P gene mRNA by the programmed insertion of G residues into a short G run contained within a larger purine run, via **pseudo**-templated transcription. The templates for paramyxovirus transcription are genome **nucleocapsids** in which each nucleoprotein subunit is associated with 6 nt, and only genomes whose lengths are multiples of 6 are found naturally or are replicated efficiently in transfected cell systems. We have examined the effect of varying total genome length on the frequency and number of insertions into the mRNA editing site in a transfected cell system, using constructs that generate mini-genome analogues. We found that, as long as the purine run sequence and the region immediately upstream were unaltered, editing occurred during mRNA synthesis independent of the precise length of the minigenome. However, when mini-genome constructs whose lengths were not multiples of 6 were used, insertions (or deletions) occurred during antigenome synthesis within the purine run, which strikingly restored the hexamer length. Genome length correction due to changes in the antigenome purine run length occurred only when the mini-genome was not a multiple of 6, and these changes were only poorly affected by mutations in the mRNA editing site and the region immediately upstream. Our results suggest that the mRNA editing site is a natural hotspot for viral polymerase slippage during genome replication, and that this site serves the dual and complementary function of maintaining hexamer genome length. The unusual requirement of paramyxoviruses for genomes of precise hexamer length may have evolved to maintain genome stability against insertions in the mRNA editing site during replication.

L8 ANSWER 11 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 1995:156639 BIOSIS  
 DOCUMENT NUMBER: PREV199598170939  
 TITLE: A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new mutations that map to the ectodomain of the E2 glycoprotein.  
 AUTHOR(S): Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)  
 CORPORATE SOURCE: (1) Dep. Mol. Microbiol., Box 8230, Washington Univ. Sch. Med., St. Louis, MO 63110-1073 USA  
 SOURCE: Virology, (1995) Vol. 206, No. 2, pp. 1027-1034. ISSN: 0042-6822.  
 DOCUMENT TYPE: Article  
 LANGUAGE: English  
 TI A **pseudo**-revertant of a Sindbis virus 6K protein mutant, which corrects for aberrant particle formation, contains two new

mutations that map to the ectodomain of the E2 glycoprotein.

SO Virology, (1995) Vol. 206, No. 2, pp. 1027-1034.  
ISSN: 0042-6822.

AU Ivanova, Lidia; Lustig, Shlomo; Schlesinger, Milton (1)

AB Most site-directed mutations in the gene encoding the small, membrane-associated 6K protein of Sindbis **virus** interfere selectively with **virus** assembly and budding. Particles are released that are aberrant in structure, with a single membrane enclosing multiple **nucleocapsids**. A revertant for the mutation that inserted a serine for a cysteine at position 39 in the 6K protein was isolated and found to correct for the defective budding so that normal particles were formed. Genetic analysis of this revertant showed that two additional mutations, which were mapped to the ectodomain of the E2 **virus** glycoprotein, were present in addition to the original 6K substitution. The phenotype of the revertant differed from the wild-type strain and the original mutation with regard to plaque size, thermostability, and growth in neuronal calls. Five new **virus** genetic constructs were prepared by insertion of these mutations into the wild-type **virus**. Phenotypes of these constructs confirmed that the mutations in the E2 ectodomain were responsible for both correcting the original defect in budding as well as imparting changes in cell tropism, plaque size, and thermolability on the **virus**. These results indicate that 6K may play an indirect role in the packing of the **virus** spike glycoproteins, which allows for membrane deformation and bending during the budding process.

L8 ANSWER 12 OF 12 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1993:327067 BIOSIS

DOCUMENT NUMBER: PREV199396035417

TITLE: Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating **virus** (LDV).

AUTHOR(S): Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)

CORPORATE SOURCE: (1) Dep. Biol., P.O. Box 4010, Georgia State Univ., Atlanta, GA 30302 USA

SOURCE: Virology, (1993) Vol. 194, No. 2, pp. 585-596.  
ISSN: 0042-6822.

DOCUMENT TYPE: Article

LANGUAGE: English

TI Complete genomic sequence and phylogenetic analysis of the lactate dehydrogenase-elevating **virus** (LDV).

SO Virology, (1993) Vol. 194, No. 2, pp. 585-596.  
ISSN: 0042-6822.

AU Godeny, E. K.; Chen, L.; Kumar, S. N.; Methven, S. L.; Koonin, E. V.; Brinton, M. A. (1)

AB The apparently complete sequence of the RNA genome of the neurovirulent isolate of lactate dehydrogenase-elevating **virus** (LDV-C) has been determined. The LDV-C genome is at least 14,222 nucleotides in length and contains eight open reading frames (ORFs). ORF 1a, which encodes a protein of 242.8 kDa and is located at the 5' end of the genome, contains at least two putative papain-like cysteine protease domains, and one putative chymotrypsin-like serine protease domain. This ORF terminates with a UAG stop codon that can be bypassed if a -1 frameshift occurs. The frameshift region consists of a heptanucleotide "slippery" sequence, 5'-UUUAAAC-3', followed by a putative **pseudo-knot**. ORF 1b encodes a protein of 155.4 kDa containing, in its N-terminal portion, an RNA-dependent RNA polymerase and an RNA helicase domain separated by a Zn finger domain. Another domain of unknown function that is also conserved in coronaviruses and toroviruses is located at the C-terminus of the ORF

1b product. Three cleavage sites in the ORF 1a polyprotein and three in the ORF 1b polyprotein were predicted for the chymotrypsin-like protease and tentatively delimit the mature nonstructural proteins of LDV. Six small, overlapping 3' ORFs (ORFs 2 through 7) encode proteins with calculated sizes of 25.8, 21.6, 19.8, 23.9, 18.9, and 12.3 kDa. ORF 7 encodes the virion **nucleocapsid** protein Vp-1, while ORF 6 encodes the nonglycosylated envelope protein Vp2. ORFs 5, 4, 3, and 2 each encode glycoproteins which may be virion envelope proteins. LDV is closely related to equine arteritis **virus**, Lelystad **virus** (LV), and simian hemorrhagic fever **virus**. These four **viruses** belong to a new group of positive-strand RNA **viruses** and are related to coronaviruses and toroviruses.

=> log off

ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF  
LOGOFF? (Y)/N/HOLD:y  
STN INTERNATIONAL LOGOFF AT 07:38:29 ON 31 OCT 2002

## WEST Search History

DATE: Thursday, October 31, 2002

<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ</i>			
L6	tRNA and L4	11	L6
L5	tRNA and L4L4	0	L5
L4	L1 and L2	78	L4
L3	L1 and L2L2	0	L3
L2	viral adj capsid	903	L2
L1	(virus like particle)	741	L1

END OF SEARCH HISTORY

## WEST Search History

DATE: Thursday, October 31, 2002

<u>Set Name</u> side by side	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u> result set
<i>DB=USPT,PGPB,JPAB,EPAB,DWPI; THES=ASSIGNEE; PLUR=YES; OP=ADJ</i>			
L9	capsid and L7	3	L9
L8	L7 and L2	0	L8
L7	crystalization	1518	L7
L6	tRNA and L4	11	L6
L5	tRNA and L4L4	0	L5
L4	L1 and L2	78	L4
L3	L1 and L2L2	0	L3
L2	viral adj capsid	903	L2
L1	(virus like particle)	741	L1

END OF SEARCH HISTORY



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=> " hepatitis C virus like particle"
    35919 "HEPATITIS"
    2959846 "C"
    274408 "VIRUS"
    52496 "VIRUSES"
    283713 "VIRUS"
        ("VIRUS" OR "VIRUSES")
    541007 "LIKE"
    173 "LIKES"
    541156 "LIKE"
        ("LIKE" OR "LIKES")
    551164 "PARTICLE"
    623424 "PARTICLES"
    938491 "PARTICLE"
        ("PARTICLE" OR "PARTICLES")
L5      11 " HEPATITIS C VIRUS LIKE PARTICLE"
        ("HEPATITIS" (W) "C" (W) "VIRUS" (W) "LIKE" (W) "PARTICLE")

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=> core and L5
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    51907 CORES
    256566 CORE
        (CORE OR CORES)
L6      3 CORE AND L5

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=> tRNA and L5
    26759 TRNA
    5314 TRNAS
    27433 TRNA
        (TRNA OR TRNAS)
L7      0 TRNA AND L5

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=> tRNA and L5
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    5314 TRNAS
    27433 TRNA
        (TRNA OR TRNAS)
L8      0 TRNA AND L5

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=> nucleocapsid and L5
    3837 NUCLEOCAPSID
    902 NUCLEOCAPSIDS
    4249 NUCLEOCAPSID
        (NUCLEOCAPSID OR NUCLEOCAPSIDS)
L9      0 NUCLEOCAPSID AND L5

```

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=> DIS L6 1- TI
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):N

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=> DIS L6 1- IBIB ABS
YOU HAVE REQUESTED DATA FROM 3 ANSWERS - CONTINUE? Y/(N):Y
THE ESTIMATED COST FOR THIS REQUEST IS 6.87 U.S. DOLLARS
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

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L6  ANSWER 1 OF 3  CAPLUS  COPYRIGHT 2002 ACS
ACCESSION NUMBER:      2002:248535  CAPLUS
DOCUMENT NUMBER:       136:398351
TITLE:                 Hepatitis C virus-
                        like particle morphogenesis

```

AUTHOR(S): Blanchard, Emmanuelle; Brand, Denys; Trassard, Sylvie;  
 CORPORATE SOURCE: Goudeau, Alain; Roingeard, Philippe  
 LABORATOIRE DE VIROLOGIE, E3M-EA3250, IFR 82, FACULTE DE MEDECINE, CENTRE HOSPITALIER UNIVERSITAIRE, TOURS, 37032, FR.  
 SOURCE: Journal of Virology (2002), 76(8), 4073-4079  
 CODEN: JOVIAM; ISSN: 0022-538X  
 PUBLISHER: American Society for Microbiology  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB Although much is known about the hepatitis C virus (HCV) genome, first cloned in 1989, little is known about HCV structure and assembly due to the lack of an efficient in vitro culture system for HCV. Using a recombinant Semliki forest virus replicon expressing genes encoding HCV structural proteins, we obsd. for the first time the assembly of these proteins into HCV-like particles in mammalian cells. This system opens up new possibilities for the investigation of viral morphogenesis and virus-host cell interactions.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
 FORMAT

L6 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:344513 CAPLUS

DOCUMENT NUMBER: 129:24164

TITLE: Synthesis and purification of **hepatitis C virus-like particles** from insect cells using a baculovirus vector

INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.

PATENT ASSIGNEE(S): United States Dept. of Health and Human Services, USA;

Liang, T. Jake; Baumert, Thomas F.

SOURCE: PCT Int. Appl., 31 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001504337	T2	20010403	JP 1998-522521	19970325

US 6387662 B1 20020514 US 1999-296441 19990421  
PRIORITY APPLN. INFO.: US 1996-30238P P 19961108  
WO 1997-US5096 W 19970325

AB Prodn. of enveloped RNA virus-like particles intracellularly in vitro in insect cells using a recombinant baculovirus vector contg. a cDNA coding for viral structural proteins is disclosed. In vitro prodn. and purifn. of hepatitis C virus (HCV)-like particles contg. HCV core protein, E1 protein and E2 protein is disclosed. Prodn. of antibodies in vivo to the purified HCV-like particles is disclosed.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:402184 CAPLUS

DOCUMENT NUMBER: 117:2184

TITLE: Non-A, Non-B hepatitis virus particle manufacture by expression of cloned cDNA

INVENTOR(S): Okayama, Hiroto; Fuke, Isao; Mori, Chisato; Takamizawa, Akihisa; Yoshida, Iwao

PATENT ASSIGNEE(S): Research Foundation for Microbial Diseases, Osaka University, Japan

SOURCE: Eur. Pat. Appl., 89 pp.

CODEN: EPXXDW

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 463848	A2	19920102	EP 1991-305717	19910625
EP 463848	A3	19920304		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AU 9168608	A1	19920102	AU 1991-68608	19910104
AU 650634	B2	19940630		
CN 1057861	A	19920115	CN 1991-100571	19910131
CA 2045326	AA	19911226	CA 1991-2045326	19910624
FI 9103068	A	19911226	FI 1991-3068	19910624
NO 9102459	A	19911227	NO 1991-2459	19910624
AU 9179256	A1	19920102	AU 1991-79256	19910624
AU 652941	B2	19940915		
CN 1059758	A	19920325	CN 1991-105293	19910625
HU 60773	A2	19921028	HU 1991-2118	19910625
JP 07258290	A2	19951009	JP 1991-180260	19910625
US 5747339	A	19980505	US 1994-324977	19941018
US 5847101	A	19981208	US 1995-384616	19950206
US 5998130	A	19991207	US 1997-904686	19970801
US 6217872	B1	20010417	US 1999-315850	19990521
PRIORITY APPLN. INFO.:				
			JP 1990-167466	A 19900625
			JP 1990-230921	A 19900831
			JP 1990-305605	A 19901109
			US 1990-635451	A 19901228
			JP 1991-132090	A 19910508
			JP 1991-138493	A 19910514
			US 1991-769996	B3 19911002
			US 1993-99706	B1 19930730
			US 1994-324977	A3 19941018
			US 1997-904686	A3 19970801

AB CDNAs derived from a large segment of the hepatitis C virus genomic RNA

are cloned and expressed to yield viral particles suitable for use in vaccines and as diagnostic reagents. The particles include at least one antigen from the **core**, matrix, or envelope. Whole RNA was prepd. from plasma or liver of hepatitis C virus-infected patients, reverse transcribed and amplified by polymerase chain reaction by std. methods and cloned into .lambda.gt11 or pUC19. Banks were screened by immune screening with serum from infected patients. Individual clones were used to manuf. antigens in Escherichia coli. Individual cDNAs were ligated together to generate a single cDNA covering much of the viral genome. This cDNA was introduced into expression vectors for bacteria, yeast and animal cells. Human hepatocytes infected with a vaccinia virus-based expression vector carrying the cDNA yielded **hepatitis C virus-like particles** when obsd. in the electron microscope.

=> DIS L5 1- IBIB ABS

YOU HAVE REQUESTED DATA FROM 11 ANSWERS - CONTINUE? Y/(N):Y  
THE ESTIMATED COST FOR THIS REQUEST IS 25.18 U.S. DOLLARS  
DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L5 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:674085 CAPLUS  
TITLE: Erratum: analysis of antigenicity and topology of E2 glycoprotein present on recombinant **hepatitis C virus-like particles**  
AUTHOR(S): Clayton, Reginald F.; Owsianka, Ania; Aitken, Jim; Graham, Susan; Bhella, David; Patel, Arvind H.  
CORPORATE SOURCE: MRC Virology Unit, Institute of Virology, University of Glasgow, Glasgow, G11 5JR, UK  
SOURCE: Journal of Virology (2002), 76(18), 9562  
CODEN: JOVIAM; ISSN: 0022-538X  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal; Errata  
LANGUAGE: English  
AB Unavailable

L5 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:674061 CAPLUS  
TITLE: Interaction of **hepatitis C virus-like particles** and cells: a model system for studying viral binding and entry  
AUTHOR(S): Triyatni, Miriam; Saunier, Bertrand; Maruvada, Padma; Davis, Anthony R.; Ulianich, Luca; Heller, Theo; Patel, Arvind; Kohn, Leonard D.; Liang, T. Jake  
CORPORATE SOURCE: Liver Diseases Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, 20892, USA  
SOURCE: Journal of Virology (2002), 76(18), 9335-9344  
CODEN: JOVIAM; ISSN: 0022-538X  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB **Hepatitis C virus-like particles** (HCV-LPs) contg. the structural proteins of HCV H77 strain (1a genotype) was used as a model for HCV virion to study

virus-cell interaction. HCV-LPs showed a buoyant d. of 1.17 to 1.22 g/cm<sup>3</sup> in a sucrose gradient and formed double-shelled particles 35 to 49 nm in diam. Flow cytometry anal. by an indirect method (detection with anti-E2 antibody) and a direct method (use of dye-labeled HCV-LPs) showed that HCV-LPs binds to several human hepatic (primary hepatocytes, HepG2, HuH7, and NKNT-3) and T-cell (Molt-4) lines. HCV-LPs binding to cells occurred in a dose- and calcium-dependent manner and was not mediated by CD81. Scatchard plot anal. suggests the presence of two binding sites for HCV-LPs with high (K<sub>d</sub> .apprx.1 .mu.g/mL) and low (K<sub>d</sub> .apprx.50 to 60 .mu.g/mL) affinities of binding. Anti-E1 and -E2 antibodies inhibited HCV-LPs binding to cells. While preincubation of HCV-LPs with very-low-d.

lipoprotein (VLDL), low-d. lipoprotein (LDL), or high-d. lipoprotein (HDL) blocked its binding to cells, preincubation of cells with VLDL, LDL, HDL, or anti-LDL-R antibody did not. Confocal microscopy anal. showed that, after binding to cells, dye-labeled HCV-LPs were internalized into the cytoplasm. This process could be inhibited with anti-E1 or anti-E2 antibodies, suggesting that E1 and E2 proteins mediate HCV-LPs binding and, subsequently, their entry into cells. Altogether, our results indicate that HCV-LPs can be used to further characterize the mechanisms involved in the early steps of HCV infection.

REFERENCE COUNT: 55 THERE ARE 55 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L5 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:536918 CAPLUS

DOCUMENT NUMBER: 137:244389

TITLE: Analysis of antigenicity and topology of E2 glycoprotein present on recombinant hepatitis C virus-like particles

AUTHOR(S): Clayton, Reginald F.; Owsianka, Ania; Aitken, Jim; Graham, Susan; Bhella, David; Patel, Arvind H.

CORPORATE SOURCE: MRC Virology Unit, Institute of Virology, University of Glasgow, Glasgow, G11 5JR, UK

SOURCE: Journal of Virology (2002), 76(15), 7672-7682  
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Purifn. of hepatitis C virus (HCV) from sera of infected patients has proven elusive, hampering efforts to perform structure-function anal. of the viral components. Recombinant forms of the viral glycoproteins have been used instead for functional studies, but uncertainty exists as to whether they closely mimic the virion proteins. Here, we used HCV virus-like particles (VLPs) generated in insect cells infected with a recombinant baculovirus expressing viral structural proteins. Electron microscopic anal. revealed a population of pleomorphic VLPs that were at least partially enveloped with bilayer membranes and had viral glycoprotein spikes protruding from the surface. Immunogold labeling using specific monoclonal antibodies (MAbs) demonstrated these protrusions

to be the E1 and E2 glycoproteins. A panel of anti-E2 MAbs was used to probe the surface topol. of E2 on the VLPs and to compare the antigenicity

of the VLPs with that of truncated E2 (E2660) or the full-length (FL) E1E2

complex expressed in mammalian cells. While most MAbS bound to all forms of antigen, a no. of others showed striking differences in their abilities

to recognize the various E2 forms. All MAbS directed against hypervariable region 1 (HVR-1) recognized both native and denatured E2660 with comparable affinities, but most bound either weakly or not at all to the FL E1E2 complex or to VLPs. HVR-1 on VLPs was accessible to these MAbS only after denaturation. Importantly, a subset of MAbS specific for amino acids 464 to 475 and 524 to 535 recognized E2660 but not VLPs or FL E1E2 complex. The antigenic differences between E2660, FL E1E2, and VLPs strongly point to the existence of structural differences, which may have functional relevance. Trypsin treatment of VLPs removed the N-terminal part of E2, resulting in a 42-kDa fragment. In the presence of detergent,

this was further reduced to a trypsin-resistant 25-kDa fragment, which could be useful for structural studies.

REFERENCE COUNT: 64 THERE ARE 64 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L5 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:496756 CAPLUS

DOCUMENT NUMBER: 137:123775

TITLE: Structural Features of Envelope Proteins on  
**Hepatitis C Virus-**

**like Particles** as Determined by  
Anti-envelope Monoclonal Antibodies and CD81 Binding  
AUTHOR(S): Triyatni, Miriam; Vergalla, John; Davis, Anthony R.;  
Hadlock, Kenneth G.; Fong, Steven K. H.; Liang, T.  
Jake

CORPORATE SOURCE: Liver Diseases Section, National Institute of  
Diabetes

and Digestive and Kidney Diseases, NIH, Bethesda, MD,  
20892, USA

SOURCE: Virology (2002), 298(1), 124-132

CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Elsevier Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The envelope glycoprotein E2 of hepatitis C virus (HCV) is a major component of the viral envelope. Knowledge of its topol. features and antigenic determinants in virions is crucial in understanding the viral binding sites to cellular receptor(s) and the induction of neutralizing antibodies. The lack of a robust cell culture system for virus propagation has hampered the characterization of E2 presented on the virion. Here the authors report the structural features of **hepatitis C virus-like particles** (HCV-LPs) of the 1a and 1b genotypes as detd. by various mouse and human monoclonal anti-envelope antibodies. Our results show that the E2 protein of HCV-LPs reacts with human monoclonal antibodies recognizing conformational determinants. Monoclonal antibodies (mAbs) specific for the hypervariable region 1 (HVR-1) sequence reacted strongly with HCV-LPs, suggesting that the HVR-1 is exposed on the viral surface. Several mAbs recognized both HCV-LPs with equally high affinity, indicating that the corresponding epitopes [amino acids (aa) 192-217 of

E1

and aa 412-423, aa 522-531, and aa 640-653 of E2] are conserved in both genotypes and exposed on the surface of the HCV-LP. The E2 and E1/E2 dimers of 1a bound strongly to the recombinant large extracellular loop

(LEL) of CD81 (CD81-LEL) of human and African green monkey, while the HCV-LP of 1a bound weakly to human CD81-LEL. E1/E2 dimers and the HCV-LPs

of 1b did not bind CD81-LEL, consistent with the notion that CD81 recognition by E2 is strain-specific and does not correlate with permissiveness of infection. A model of the topol. and exposed antigenic determinants of the envelope proteins of HCV is proposed.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L5 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:248535 CAPLUS

DOCUMENT NUMBER: 136:398351

TITLE: **Hepatitis C virus-like particle** morphogenesis

AUTHOR(S): Blanchard, Emmanuelle; Brand, Denys; Trassard, Sylvie;

Goudeau, Alain; Roingeard, Philippe  
CORPORATE SOURCE: Laboratoire de Virologie, E3M-EA3250, IFR 82, Faculte de Medecine, Centre Hospitalier Universitaire, Tours, 37032, Fr.

SOURCE: Journal of Virology (2002), 76(8), 4073-4079

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Although much is known about the hepatitis C virus (HCV) genome, first cloned in 1989, little is known about HCV structure and assembly due to the lack of an efficient in vitro culture system for HCV. Using a recombinant Semliki forest virus replicon expressing genes encoding HCV structural proteins, we obsd. for the first time the assembly of these proteins into HCV-like particles in mammalian cells. This system opens

up  
new possibilities for the investigation of viral morphogenesis and virus-host cell interactions.

REFERENCE COUNT: 27 THERE ARE 27 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L5 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:79471 CAPLUS

DOCUMENT NUMBER: 136:260957

TITLE: Binding of **hepatitis C virus-like particles**

derived from infectious clone H77C to defined human cell lines

AUTHOR(S): Wellnitz, Sabine; Klumpp, Bettina; Barth, Heidi; Ito, Susumu; Depla, Erik; Dubuisson, Jean; Blum, Hubert E.;

Baumert, Thomas F.  
CORPORATE SOURCE: Department of Medicine II, University of Freiburg, Freiburg, D-79106, Germany

SOURCE: Journal of Virology (2002), 76(3), 1181-1193

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Hepatitis C virus (HCV) is a leading cause of chronic hepatitis in the world. The study of viral entry and infection has been hampered by the inability to efficiently propagate the virus in cultured cells and the lack of a small-animal model. Recent studies have shown that in insect cells, the HCV structural proteins assemble into HCV-like particles (HCV-LPs) with morphol., biophys., and antigenic properties similar to those of putative virions isolated from HCV-infected humans. In this study, we used HCV-LPs derived from infectious clone H77C as a tool to examine virus-cell interactions. The binding of partially purified particles to human cell lines was analyzed by fluorescence-activated cell sorting with defined monoclonal antibodies to envelope glycoprotein E2. HCV-LPs demonstrated dose-dependent and saturable binding to defined

human

lymphoma and hepatoma cell lines but not to mouse cell lines. Binding could be inhibited by monoclonal anti-E2 antibodies, indicating that the HCV-LP-cell interaction was mediated by envelope glycoprotein E2.

Binding

appeared to be CD81 independent and did not correlate with low-d. lipoprotein receptor expression. Heat denaturation of HCV-LPs

drastically

reduced binding, indicating that the interaction of HCV-LPs with target cells was dependent on the proper conformation of the particles. In conclusion, our data demonstrate that insect cell-derived HCV-LPs bind specifically to defined human cell lines. Since the envelope proteins of HCV-LPs are presumably presented in a virion-like conformation, the binding of HCV-LPs to target cells may allow the study of virus-host cell interactions, including the isolation of HCV receptor candidates and antibody-mediated neutralization of binding.

REFERENCE COUNT: 56 THERE ARE 56 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L5 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:605968 CAPLUS

DOCUMENT NUMBER: 136:198509

TITLE: **Hepatitis C virus-like particles** induce virus-specific humoral and cellular immune responses in mice

AUTHOR(S): Lechmann, Martin; Murata, Kazumoto; Sato, Jujin; Vergalla, John; Baumert, Thomas F.; Liang, T. Jake

CORPORATE SOURCE: Liver Diseases Section, NIDDK, National Institutes of Health, Bethesda, MD, 20892, USA

SOURCE: Hepatology (Philadelphia, PA, United States) (2001), 34(2), 417-423

CODEN: HPTLD9; ISSN: 0270-9139

PUBLISHER: W. B. Saunders Co.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB We have recently described the prodn. of **hepatitis C virus-like particles** (HCV-LPs) in insect cells that resemble the putative virions. Here we evaluate the humoral and cellular immunogenicity of the virus-like particles with or without viral p7 protein, a small viral polypeptide that resides between the structural and nonstructural regions of the HCV polyprotein and whose function has not been defined. Immunized BALB/c mice developed high titers of anti-E2 antibodies and virus-specific cellular immune responses including cytotoxic T lymphocytes and T helper responses with gamma interferon prodn. The virus-like particles without p7 generated a higher cellular immune response with a more TH1 profile than the particles with p7.



Immunization of heat-denatured particles resulted in substantially lower humoral and cellular responses, suggesting that the immunogenicity is strongly dependent on particle formation. Administration of CpG oligonucleotide or cationic lipid 3.beta.-[N-(N',N'-dimethylaminoethane)carbamoyl]-cholesterol (DC-Chol), two potent adjuvants, did not significantly enhance the immunogenicity of HCV-LPs. These results indicate that HCV-LPs can induce humoral and cellular immune

responses and offer a promising approach to vaccine development.  
 REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
 FORMAT

L5 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:396894 CAPLUS

DOCUMENT NUMBER: 135:18547

TITLE: Virus-like particles for vaccination against hepatitis

INVENTOR(S): Selby, Mark; Glazer, Edward; Houghton, Michael

PATENT ASSIGNEE(S): Chiron Corporation, USA

SOURCE: PCT Int. Appl., 115 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001038358	A2	20010531	WO 2000-US32249	20001122
WO 2001038358	A3	20020110		
WO 2001038358	C2	20020627		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
EP 1233783	A2	20020828	EP 2000-982225	20001122
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			

PRIORITY APPLN. INFO.: US 1999-167224P A2 19991124  
 WO 2000-US32249 W 20001122

AB The authors disclose the construction of chimeric antigens derived from hepatitis B virus (HBV) and hepatitis C virus (HCV) which form virus-like particles when co-expressed with an excess of hepatitis B virus surface antigen (HBsAg). The chimeric antigens are fusion proteins contg. an immunogenic peptide derived from HCV coupled to the N-terminus of HBsAg. Also described are nucleic acid constructs and vectors for transfection of cells and expression of the chimeric antigens. The authors also disclose methods for producing HBV/HCV virus-like particles contg. the chimeric antigens, cell lines for producing the virus-like particles, combination vaccines contg. the virus-like particles, and DNA vaccines that express the virus-like particles.

L5 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:659593 CAPLUS

DOCUMENT NUMBER: 134:221173

TITLE: Antibodies against **hepatitis C virus-like particles** and

virial clearance in acute and chronic hepatitis C  
AUTHOR(S): Baumert, Thomas F.; Wellnitz, Sabine; Aono, Shigeaki;  
Satoi, Jujin; Herion, David; Gerlach, Tilman; Pape,  
Gerd R.; Lau, Johnson Y. N.; Hoofnagle, Jay H.; Blum,  
Hubert E.; Liang, T. Jake

CORPORATE SOURCE: Department of Medicine II, University of Freiburg  
Medical School, Freiburg, D-79106, Germany

SOURCE: Hepatology (Philadelphia) (2000), 32(3), 610-617  
CODEN: HPTL9; ISSN: 0270-9139

PUBLISHER: W. B. Saunders Co.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The authors recently described the efficient assembly of hepatitis C  
virus

(HCV) structural proteins into HCV-like particles (HCV-LPs) in insect cells. These noninfectious HCV-LPs have similar morphol. and biophys. properties as putative virions isolated from HCV-infected humans and can induce a broadly directed immune response in animal models. The HCV envelope proteins of HCV-LPs are presumably presented in a native, virion-like conformation and may therefore interact with anti-envelope antibodies directed against conformational epitopes. Here, HCV-LPs were used as capture antigens in an ELISA to detect and quantify antibodies against HCV structural proteins in patients with acute and chronic hepatitis C. High titers of anti-HCV-LP antibodies were detected in patients chronically infected with HCV genotypes 1-6. In contrast to individuals with chronic hepatitis C, patients with acute self-limited hepatitis C displayed only a transient and weak seroreactivity against HCV-LPs. Patients with chronic HCV infection successfully treated with interferon demonstrated a gradual decline of anti-HCV-LP titers during or subsequent to viral clearance. Sustained interferon responders were characterized by higher pretreatment levels of anti-HCV-LP antibodies as compared with nonresponders. Thus, HCV infection is assocd. with limited humoral immunity against the envelope proteins present on the HCV-LPs.

An

HCV-LP-based ELISA may be a useful diagnostic tool to distinguish acute hepatitis C from chronic HCV infection with exacerbation, and to predict viral clearance in response to interferon.

REFERENCE COUNT: 42 THERE ARE 42 CITED REFERENCES AVAILABLE FOR  
THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE  
FORMAT

L5 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:344513 CAPLUS

DOCUMENT NUMBER: 129:24164

TITLE: Synthesis and purification of **hepatitis C virus-like particles** from insect cells using a baculovirus vector

INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.

PATENT ASSIGNEE(S): United States Dept. of Health and Human Services,  
USA;

SOURCE: Liang, T. Jake; Baumert, Thomas F.

PCT Int. Appl., 31 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent  
 LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001504337	T2	20010403	JP 1998-522521	19970325
US 6387662	B1	20020514	US 1999-296441	19990421
PRIORITY APPLN. INFO.:			US 1996-30238P	P 19961108
			WO 1997-US5096	W 19970325
AB Prodn. of enveloped RNA virus-like particles intracellularly in vitro in insect cells using a recombinant baculovirus vector contg. a cDNA coding for viral structural proteins is disclosed. In vitro prodn. and purifn. of hepatitis C virus (HCV)-like particles contg. HCV core protein, E1 protein and E2 protein is disclosed. Prodn. of antibodies in vivo to the purified HCV-like particles is disclosed.				
REFERENCE COUNT:		4	THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE	
FORMAT				

L12 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:478160 CAPLUS  
DOCUMENT NUMBER: 121:78160  
TITLE: Hepatitis C virus particle detected by immunoelectron  
microscopic study  
AUTHOR(S): Kaito, Masahiko; Watanabe, Shozo; Tsukiyama-Kohara,  
Kyoko; Yamaguchi, Kenjiro; Kobayashi, Yoshinao;  
Konishi, Masayoshi; Yokoi, Masato; Ishida, Satoshi;  
Suzuki, Shiro; Kohara, Michinori  
CORPORATE SOURCE: Sch. Med., Mie Univ., Mie, 514, Japan  
SOURCE: Journal of General Virology (1994), 75(7), 1755-60  
CODEN: JGVIAI; ISSN: 0022-1317  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB To clarify the morphol. of hepatitis C virus (HCV), an indirect  
immunogold

electron microscope study was carried out on two plasma samples with high  
HCV RNA titers using polyclonal and monoclonal antibodies specific to the  
putative HCV envelope protein. Spherical **virus-like  
particles** 55 to 65 nm in diam. with spike-like projections, were  
found in 1.14 to 1.6 g/mL fractions after sucrose d. gradient  
centrifugation. These particles were found only in HCV-infected blood  
donors and had morphol. features similar to those of **flaviviruses**  
. Moreover, these particles specifically reacted with the polyclonal and  
monoclonal antibodies to the putative HCV envelope protein. This is the  
first known report in which the morphol. of the HCV particle is clearly  
shown.

L12 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:55842 CAPLUS  
DOCUMENT NUMBER: 118:55842  
TITLE: Regulation of the late events in **flavivirus**  
protein processing and maturation  
AUTHOR(S): Yamshchikov, Vladimir F.; Compans, Richard W.  
CORPORATE SOURCE: Dep. Microbiol., Univ. Alabama, Birmingham, AL,  
35294,  
USA  
SOURCE: Virology (1992), 192(1), 38-51  
CODEN: VIRLAX; ISSN: 0042-6822  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB In order to det. the requirements for secretion of **flavivirus**  
structural proteins, the authors analyzed the expression of several West  
Nile **flavivirus** gene cassettes of different lengths in vaccinia  
virus expression systems. Expression of the longest cassette coding for  
the 5'-nontranslated region, proteins C through NS2B, and the protease  
domain of NS3, resulted in secretion of prM-E complexes and cleavage of  
prM. The presence and proper processing of the NS2A-NS2B-NS3 region  
appeared to be necessary for prM-E secretion. These proteins were  
released from cells mostly as membranous complexes which may represent  
empty viral envelopes. Cleavage of the membrane-assocd. intracellular  
form of protein C (Ci) to produce the virion form (Ce) appeared to be  
crit. for release of viral proteins. The presence and proper cleavage of  
the NS2A-NS2B-NS3 region were also found to be necessary for efficient  
C-prM cleavage by signalases. The NS2B-NS3 complex was implicated in  
cleavage of the intracellular form of protein C. Formation of a low  
level

of **virus-like particles** was detected by  
electron microscopy. A model for virion formation, suggesting a crit.

role of the NS2B and NS3 proteins, is discussed.

=> DIS L2 1 IBIB ABS

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DO YOU WANT TO CONTINUE WITH THIS REQUEST? (Y)/N:Y

L2 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2001:396894 CAPLUS

DOCUMENT NUMBER: 135:18547

TITLE: Virus-like particles for vaccination against hepatitis

INVENTOR(S): Selby, Mark; Glazer, Edward; Houghton, Michael

PATENT ASSIGNEE(S): Chiron Corporation, USA

SOURCE: PCT Int. Appl., 115 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001038358	A2	20010531	WO 2000-US32249	20001122
WO 2001038358	A3	20020110		
WO 2001038358	C2	20020627		
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
EP 1233783	A2	20020828	EP 2000-982225	20001122
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR			

PRIORITY APPLN. INFO.: US 1999-167224P A2 19991124  
WO 2000-US32249 W 20001122

AB The authors disclose the construction of chimeric antigens derived from hepatitis B virus (HBV) and hepatitis C virus (HCV) which form virus-like particles when co-expressed with an excess of hepatitis B virus surface antigen (HBsAg). The chimeric antigens are fusion proteins contg. an immunogenic peptide derived from HCV coupled to the N-terminus of HBsAg. Also described are nucleic acid constructs and vectors for transfection of cells and expression of the chimeric antigens. The authors also disclose methods for producing HBV/HCV virus-like particles contg. the chimeric antigens, cell lines for producing the virus-like particles, combination vaccines contg. the virus-like particles, and DNA vaccines that express the virus-like particles.

L5 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1992:402184 CAPLUS  
DOCUMENT NUMBER: 117:2184  
TITLE: Non-A, Non-B hepatitis virus particle manufacture by  
expression of cloned cDNA  
INVENTOR(S): Okayama, Hiroto; Fuke, Isao; Mori, Chisato;  
Takamizawa, Akihisa; Yoshida, Iwao  
PATENT ASSIGNEE(S): Research Foundation for Microbial Diseases, Osaka  
University, Japan  
SOURCE: Eur. Pat. Appl., 89 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 2  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
-----	----	-----	-----	-----
EP 463848	A2	19920102	EP 1991-305717	19910625
EP 463848	A3	19920304		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AU 9168608	A1	19920102	AU 1991-68608	19910104
AU 650634	B2	19940630		
CN 1057861	A	19920115	CN 1991-100571	19910131
CA 2045326	AA	19911226	CA 1991-2045326	19910624
FI 9103068	A	19911226	FI 1991-3068	19910624
NO 9102459	A	19911227	NO 1991-2459	19910624
AU 9179256	A1	19920102	AU 1991-79256	19910624
AU 652941	B2	19940915		
CN 1059758	A	19920325	CN 1991-105293	19910625
HU 60773	A2	19921028	HU 1991-2118	19910625
JP 07258290	A2	19951009	JP 1991-180260	19910625
US 5747339	A	19980505	US 1994-324977	19941018
US 5847101	A	19981208	US 1995-384616	19950206
US 5998130	A	19991207	US 1997-904686	19970801
US 6217872	B1	20010417	US 1999-315850	19990521
PRIORITY APPLN. INFO.:			JP 1990-167466	A 19900625
			JP 1990-230921	A 19900831
			JP 1990-305605	A 19901109
			US 1990-635451	A 19901228
			JP 1991-132090	A 19910508
			JP 1991-138493	A 19910514
			US 1991-769996	B3 19911002
			US 1993-99706	B1 19930730
			US 1994-324977	A3 19941018
			US 1997-904686	A3 19970801

AB CDNAs derived from a large segment of the hepatitis C virus genomic RNA are cloned and expressed to yield viral particles suitable for use in vaccines and as diagnostic reagents. The particles include at least one antigen from the core, matrix, or envelope. Whole RNA was prepd. from plasma or liver of hepatitis C virus-infected patients, reverse transcribed and amplified by polymerase chain reaction by std. methods and

cloned into .lambda.gt11 or pUC19. Banks were screened by immune screening with serum from infected patients. Individual clones were used to manuf. antigens in Escherichia coli. Individual cDNAs were ligated together to generate a single cDNA covering much of the viral genome. This cDNA was introduced into expression vectors for bacteria, yeast and animal cells. Human hepatocytes infected with a vaccinia virus-based expression vector carrying the cDNA yielded **hepatitis C**

**virus-like particles** when obsd. in the  
electron microscope.



L12 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:344513 CAPLUS  
DOCUMENT NUMBER: 129:24164  
TITLE: Synthesis and purification of hepatitis C  
**virus-like particles** from  
insect cells using a baculovirus vector  
INVENTOR(S): Liang, T. Jake; Baumert, Thomas F.  
PATENT ASSIGNEE(S): United States Dept. of Health and Human Services,  
USA;  
Liang, T. Jake; Baumert, Thomas F.  
SOURCE: PCT Int. Appl., 31 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9821338	A1	19980522	WO 1997-US5096	19970325
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, EE, ES, FI, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9723479	A1	19980603	AU 1997-23479	19970325
AU 738585	B2	20010920		
EP 941337	A1	19990915	EP 1997-916252	19970325
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
JP 2001504337	T2	20010403	JP 1998-522521	19970325
US 6387662	B1	20020514	US 1999-296441	19990421
PRIORITY APPLN. INFO.:			US 1996-30238P P	19961108
			WO 1997-US5096 W	19970325

AB Prodn. of enveloped RNA **virus-like particles**  
intracellularly in vitro in insect cells using a recombinant baculovirus  
vector contg. a cDNA coding for viral structural proteins is disclosed.  
In vitro prodn. and purifn. of hepatitis C virus (HCV)-like particles  
contg. HCV core protein, E1 protein and E2 protein is disclosed. Prodn.  
of antibodies in vivo to the purified HCV-like particles is disclosed.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS  
RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L12 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:494028 CAPLUS  
DOCUMENT NUMBER: 127:202729  
TITLE: Expression of the dengue virus structural proteins in  
Pichia pastoris leads to the generation of  
**virus-like particles**  
AUTHOR(S): Sugrue, Richard Joseph; Fu, Jianlin; Howe, Josephine;  
Chan, Yow-Cheong  
CORPORATE SOURCE: Dengue Virus Group, Institute of Molecular and Cell  
Biology, National University of Singapore, Singapore  
SOURCE: Journal of General Virology (1997), 78(8), 1861-1866  
CODEN: JGVIA Y; ISSN: 0022-1317  
PUBLISHER: Society for General Microbiology

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB We have expressed cDNA encoding the dengue virus structural proteins in *Pichia pastoris* by chromosomal integration of an expression cassette contg. the dengue virus structural genes (CprME). The yeast recombinant

E protein migrated during SDS-PAGE as a 65 kDa protein when analyzed by Western blotting and radioimmunopptn., which is the expected mol. mass

for correctly processed and glycosylated E protein. Treatment with endoglycosidases showed that the recombinant E protein was modified by

the addn. of short mannose chains. The E protein migrated with a buoyant d. of 1.13 g/cm<sup>3</sup> when analyzed using sucrose d. gradient centrifugation. Spherical structures with an av. diam. of 30 nm, whose morphol. resembles dengue virions, were obsd. in the purified fractions using transmission electron microscopy. Furthermore, the **virus-like particles** were immunogenic in animals and were able to induce neutralizing antibodies. This is the first report that expression of the structural genes of a **flavivirus** in yeast is able to generate particulate structures that resemble virions.

L12 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:155646 CAPLUS

DOCUMENT NUMBER: 131:68778

TITLE: Noncytopathic **Flavivirus** Replicon RNA-Based  
System for Expression and Delivery of Heterologous  
Genes

AUTHOR(S): Varnavski, Andrei N.; Khromykh, Alexander A.

CORPORATE SOURCE: Sir Albert Sakzewski Virus Research Centre, Royal  
Children's Hospital, Brisbane, 4029, Australia

SOURCE: Virology (1999), 255(2), 366-375

CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Noncytopathic replicons of the **flavivirus** Kunjin (KUN) were employed for expression and delivery of heterologous genes. Replicon vector C20DX2Arep, contg. a unique cloning site followed by the sequence of 2A autoprotease of foot-and-mouth disease virus, was constructed and used for expression of a no. of heterologous genes including chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), .beta.-galactosidase, glycoprotein G of vesicular stomatitis virus, and the Core and NS3 genes of hepatitis C virus. The expression and proper processing of these genes upon transfection of BHK21 cells with the recombinant replicon RNAs were demonstrated by immunofluorescence, radioimmunopptn., and appropriate reporter gene assays. Most of these recombinant KUN replicon RNAs were also successfully packaged into secreted **virus-like particles (VLPs)** by subsequent transfection with Semliki Forest virus replicon RNA expressing KUN structural genes. Infection of BHK21 and Vero cells with these **VLPs** resulted in continuous replication of the recombinant replicon RNAs and prolonged expression of the cloned genes without any cytopathic effect. We also developed a replicon vector for generation of stable cell lines continuously expressing heterologous genes by inserting an encephalomyelocarditis virus internal ribosomal entry site-neomycin transferase gene cassette into the 3'-untranslated region of the C20DX2Arep vector. Using this vector (C20DX2ArepNeo), stable BHK cell lines persistently expressing GFP and CAT genes for up to 17 passages were

established. Thus noncytopathic KUN replicon vectors with the ability to be packaged into **VLPs** should provide a useful tool for the development of noninfectious and noncytopathic vaccines as well as for gene therapy applications. (c) 1999 Academic Press.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR  
THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L12 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:384227 CAPLUS  
DOCUMENT NUMBER: 133:29600  
TITLE: Capsid particles of hepatitis B core antigen for presentation of immunogenic components  
INVENTOR(S): Murray, Kenneth  
PATENT ASSIGNEE(S): Biogen, Inc., USA  
SOURCE: PCT Int. Appl., 60 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032625	A1	20000608	WO 1999-US28755	19991203
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
BR 9915942	A	20010821	BR 1999-15942	19991203
EP 1135408	A1	20010926	EP 1999-961935	19991203
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
JP 2002532387	T2	20021002	JP 2000-585266	19991203
US 2002064533	A1	20020530	US 2001-873459	20010604
NO 2001002760	A	20010806	NO 2001-2760	20010605
PRIORITY APPLN. INFO.:			US 1998-110911P P	19981204
			WO 1999-US28755 W	19991203

AB The authors discloses the use of hepatitis B virus (HBV) core antigen particles for presentation to the immune system of multiple immunogen specificities. The immunogens, epitopes, or other related structures, are crosslinked or fused to HBV capsid-binding peptides that selectively bind to HBV core protein. Mixts. of different immunogens and/or capsid-binding peptide ligands may be crosslinked to the same HBV core particle. Such resulting multicomponent or multivalent HBV core particles may be advantageously used in therapeutic and prophylactic vaccines and compns., as well as in diagnostic applications.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE

FORMAT

L13 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:55842 CAPLUS

DOCUMENT NUMBER: 118:55842

TITLE: Regulation of the late events in **flavivirus**  
protein processing and maturation

AUTHOR(S): Yamshchikov, Vladimir F.; Compans, Richard W.

CORPORATE SOURCE: Dep. Microbiol., Univ. Alabama, Birmingham, AL,  
35294,

USA

SOURCE: Virology (1992), 192(1), 38-51

CODEN: VIRLAX; ISSN: 0042-6822

DOCUMENT TYPE: Journal

LANGUAGE: English

AB In order to det. the requirements for secretion of **flavivirus** structural proteins, the authors analyzed the expression of several West Nile **flavivirus** gene cassettes of different lengths in vaccinia virus expression systems. Expression of the longest cassette coding for the 5'-nontranslated region, proteins C through NS2B, and the protease domain of NS3, resulted in secretion of prM-E complexes and cleavage of prM. The presence and proper processing of the NS2A-NS2B-NS3 region appeared to be necessary for prM-E secretion. These proteins were released from cells mostly as membranous complexes which may represent empty viral envelopes. Cleavage of the membrane-assocd. intracellular form of protein C (Ci) to produce the virion form (Ce) appeared to be crit. for release of viral proteins. The presence and proper cleavage of the NS2A-NS2B-NS3 region were also found to be necessary for efficient C-prM cleavage by signalases. The NS2B-NS3 complex was implicated in cleavage of the intracellular form of protein C. Formation of a low

level

of **virus-like particles** was detected by electron microscopy. A model for virion formation, suggesting a crit. role of the NS2B and NS3 proteins, is discussed.

L13 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:384227 CAPLUS  
DOCUMENT NUMBER: 133:29600  
TITLE: **Capsid** particles of hepatitis B core antigen  
for presentation of immunogenic components  
INVENTOR(S): Murray, Kenneth  
PATENT ASSIGNEE(S): Biogen, Inc., USA  
SOURCE: PCT Int. Appl., 60 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000032625	A1	20000608	WO 1999-US28755	19991203
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
BR 9915942	A	20010821	BR 1999-15942	19991203
EP 1135408	A1	20010926	EP 1999-961935	19991203
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
JP 2002532387	T2	20021002	JP 2000-585266	19991203
US 2002064533	A1	20020530	US 2001-873459	20010604
NO 2001002760	A	20010806	NO 2001-2760	20010605
PRIORITY APPLN. INFO.:			US 1998-110911P	P 19981204
			WO 1999-US28755	W 19991203

AB The authors discloses the use of hepatitis B virus (HBV) core antigen particles for presentation to the immune system of multiple immunogen specificities. The immunogens, epitopes, or other related structures, are crosslinked or fused to HBV **capsid**-binding peptides that selectively bind to HBV core protein. Mixts. of different immunogens and/or **capsid**-binding peptide ligands may be crosslinked to the same HBV core particle. Such resulting multicomponent or multivalent HBV core particles may be advantageously used in therapeutic and prophylactic vaccines and compns., as well as in diagnostic applications.

L16 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:155646 CAPLUS

DOCUMENT NUMBER: 131:68778

TITLE: Noncytopathic **Flavivirus** Replicon RNA-Based System for Expression and Delivery of Heterologous Genes

AUTHOR(S): Varnavski, Andrei N.; Khromykh, Alexander A.

CORPORATE SOURCE: Sir Albert Sakzewski Virus Research Centre, Royal Children's Hospital, Brisbane, 4029, Australia

SOURCE: Virology (1999), 255(2), 366-375

CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Noncytopathic replicons of the **flavivirus** Kunjin (KUN) were employed for expression and delivery of heterologous genes. Replicon vector C20DX2Arep, contg. a unique cloning site followed by the sequence of 2A autoprotease of foot-and-mouth disease virus, was constructed and used for expression of a no. of heterologous genes including chloramphenicol acetyltransferase (CAT), green fluorescent protein (GFP), .beta.-galactosidase, glycoprotein G of vesicular stomatitis virus, and the **Core** and NS3 genes of hepatitis C virus. The expression and proper processing of these genes upon transfection of BHK21 cells with

the

recombinant replicon RNAs were demonstrated by immunofluorescence, radioimmunopptn., and appropriate reporter gene assays. Most of these recombinant KUN replicon RNAs were also successfully packaged into secreted **virus-like particles (VLPs)**

) by subsequent transfection with Semliki Forest virus replicon RNA expressing KUN structural genes. Infection of BHK21 and Vero cells with these **VLPs** resulted in continuous replication of the recombinant replicon RNAs and prolonged expression of the cloned genes without any cytopathic effect. We also developed a replicon vector for generation of stable cell lines continuously expressing heterologous genes by inserting an encephalomyelocarditis virus internal ribosomal entry site-neomycin transferase gene cassette into the 3'-untranslated region of the C20DX2Arep vector. Using this vector (C20DX2ArepNeo), stable BHK cell lines persistently expressing GFP and CAT genes for up to 17 passages

were

established. Thus noncytopathic KUN replicon vectors with the ability to be packaged into **VLPs** should provide a useful tool for the development of noninfectious and noncytopathic vaccines as well as for gene therapy applications. (c) 1999 Academic Press.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS

FORMAT

RECORD. ALL CITATIONS AVAILABLE IN THE RE



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/40, C07K 14/18, A61K 39/29,</b> <b>C07K 16/10, G01N 33/569, C12N 5/10</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/21338</b> <b>(43) International Publication Date:</b> 22 May 1998 (22.05.98)
<b>(21) International Application Number:</b> PCT/US97/05096 <b>(22) International Filing Date:</b> 25 March 1997 (25.03.97) <b>(30) Priority Data:</b> 60/030,238      8 November 1996 (08.11.96)      US <b>(71) Applicant (for all designated States except US):</b> THE GOVERNMENT OF THE UNITED STATES OF AMERICA, represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; National Institutes of Health, Office of Technology Transfer, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852-3804 (US). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> LIANG, T., Jake [US/US]; 9417 Lost Trail Way, Potomac, MD 20854 (US). BAUMERT, Thomas, F. [US/US]; Apartment 2, 4867 Battery Lane, Bethesda, MD 20814 (US). <b>(74) Agent:</b> ALTMAN, Daniel, E.; Knobbe, Martens, Olson & Bear, 16th floor, 620 Newport Center Drive, Newport Beach, CA 92660 (US).		<b>(81) Designated States:</b> AL, AM, AT, AT (Utility model), AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ (Utility model), DE, DE (Utility model), DK, DK (Utility model), EE, EE (Utility model), ES, FI, FI (Utility model), GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK (Utility model), TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>With amended claims.</i>
<b>(54) Title:</b> SYNTHESIS AND PURIFICATION OF HEPATITIS C VIRUS-LIKE PARTICLES <b>(57) Abstract</b> <p>Production of enveloped RNA virus-like particles intracellularly <i>in vitro</i> in insect cells using a recombinant baculovirus vector containing a cDNA coding for viral structural proteins is disclosed. <i>In vitro</i> production and purification of hepatitis C virus (HCV)-like particles containing HCV core protein, E1 protein and E2 protein is disclosed. Production of antibodies <i>in vivo</i> to the purified HCV-like particles is disclosed.</p> <div data-bbox="682 1176 1234 1617" data-label="Image"> </div>		



## INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/05096

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/40 C07K14/18 A61K39/29 C07K16/10 G01N33/569  
C12N5/10

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HEPATOLOGY, vol. 24, no. 4, Pt.2(Supplement), October 1996, page 251A XP002036710 T.F.BAUMERT ET AL.: "Synthesis of Hepatitis C virus-like particles in insect cells" see abstract 500	1-15, 19-22
X	EP 0 577 894 A (KOREA GREEN CROSS CORPORATION) 12 January 1994 see page 6, line 46 - page 8, line 26 --- -/--	1-6



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

## \* Special categories of cited documents:

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date, but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*&\* document member of the same patent family

Date of the actual completion of the international search

4 August 1997

Date of mailing of the international search report

14.08.97

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax (+31-70) 340-3016

Authorized officer

Cupido, M

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 97/05096

## C(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JOURNAL OF GENERAL VIROLOGY, vol. 73, no. PART 07, 1 July 1992, pages 1819-1824, XP000288658 THOMSEN D R ET AL: "Expression of feline leukaemia virus gp85 and gag proteins and assembly into virus-like particles using the baculovirus expression vector system" see the whole document ---	1-5
X	WO 95 33053 A (CHIRON CORPORATION) 7 December 1995	16,17
A	see page 19, line 20 - line 27 -----	10,11

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 97/05096

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:  
Remark: Although claim(s) 5 and 6  
is(are) directed to a method of treatment of the human/animal  
body, the search has been carried out and based on the alleged  
effects of the compound/composition.
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such  
an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all  
searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment  
of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report  
covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is  
restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

(information on patent family members)

International Application No

PCT/US 97/05096

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 577894 A	12-01-94	JP 6321997 A	22-11-94
		US 5580773 A	03-12-96
		US 5443828 A	22-08-95
-----			
WO 9533053 A	07-12-95	AU 2550995 A	21-12-95
		CA 2190265 A	07-12-95
		EP 0760855 A	12-03-97
-----			

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    254992 "SELF"
        20 "SELFS"
        31 "SELVES"
    255037 "SELF"
        ("SELF" OR "SELFS" OR "SELVES")
    101096 "ASSEMBLY"
    25117 "ASSEMBLIES"
    116983 "ASSEMBLY"
        ("ASSEMBLY" OR "ASSEMBLIES")
L1    11719 "SELF-ASSEMBLY"
        ("SELF" (W) "ASSEMBLY")

=> " in vitro" and L1
    516904 "VITRO"
        70 "VITROS"
    516972 " IN VITRO"
        ("VITRO" OR "VITROS")
L2    422 " IN VITRO" AND L1

=> " cell free" and L1
    1586126 "CELL"
    1413425 "CELLS"
    2138443 "CELL"
        ("CELL" OR "CELLS")
    1071709 "FREE"
        531 "FREES"
    1072153 "FREE"
        ("FREE" OR "FREES")
    32767 " CELL FREE"
        ("CELL" (W) "FREE")
L3    18 " CELL FREE" AND L1

=> "viral like partikel" and L3
    110274 "VIRAL"
        5 "VIRALS"
    110279 "VIRAL"
        ("VIRAL" OR "VIRALS")
    541007 "LIKE"
        173 "LIKES"
    541156 "LIKE"
        ("LIKE" OR "LIKES")
    19 "PARTICEL"
    19 "PARTICLES"
    38 "PARTICEL"
        ("PARTICEL" OR "PARTICLES")
    0 "VIRAL LIKE PARTICEL"
        ("VIRAL" (W) "LIKE" (W) "PARTICEL")
L4    0 "VIRAL LIKE PARTICEL" AND L3

=> "virus like particle"
    274408 "VIRUS"
    52496 "VIRUSES"
    283713 "VIRUS"
        ("VIRUS" OR "VIRUSES")
    541007 "LIKE"
        173 "LIKES"
    541156 "LIKE"
        ("LIKE" OR "LIKES")
    551164 "PARTICLE"

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623424 "PARTICLES"  
938491 "PARTICLE"  
("PARTICLE" OR "PARTICLES")  
L5 1489 "VIRUS LIKE PARTICLE"  
("VIRUS" (W) "LIKE" (W) "PARTICLE")

=> L5 and L3

L6 2 L5 AND L3

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=> tRNA

L1 42320 TRNA

=> crystalization and L1

L2 0 CRYSTALIZATION AND L1

=> "Transfer RNA or tRNA"

L3 788 "TRANSFER RNA OR TRNA"

=> crystalization and L3

L4 0 CRYSTALIZATION AND L3

=> crystalization

L5 139 CRYSTALIZATION

=> :5 and L3

L6 224 :5 AND L3

=> L5 and L3

L7 0 L5 AND L3

=> log off

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LOGOFF? (Y)/N/HOLD:y  
STN INTERNATIONAL LOGOFF AT 12:57:34 ON 31 OCT 2002

L3 ANSWER 15 OF 18 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1988:626196 CAPLUS

DOCUMENT NUMBER: 109:226196

TITLE: In vitro assay for protein-protein interaction:  
carboxyl-terminal 40 residues of simian virus 40  
structural protein VP3 contain a determinant for  
interaction with VP1

AUTHOR(S): Gharakhanian, Editte; Takahashi, Jeffrey; Clever,  
Jared; Kasamatsu, Harumi

CORPORATE SOURCE: Mol. Biol. Inst., Univ. California, Los Angeles, CA,  
90024-1606, USA

SOURCE: Proceedings of the National Academy of Sciences of  
the

United States of America (1988), 85(18), 6607-11

CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Intermol. interactions between polypeptide chains play essential roles in  
the functioning of proteins. An in vitro assay system for identifying  
and

characterizing such interactions is described. Such interactions are  
difficult to study in vivo. Synthetic, nonmethyl-capped RNAs were  
translated in a **cell-free** protein-synthesizing system.

The translation products were allowed to interact posttranslationally to  
form protein-protein complexes. The chem. nature of the protein  
interaction(s) was detd. by coimmunopptn. of assocg. proteins,  
sedimentation through sucrose gradients, followed by SDS-polyacrylamide  
gel electrophoresis or by nonreducing SDS-polyacrylamide gel  
electrophoresis. The system has been utilized to show the **self-**  
**assembly** of monomeric VP1, the major structural protein of simian  
virus 40, into disulfide-linked pentamers and to show the noncovalent  
interaction of another structural protein, VP3, with VP1 at low monomer  
concns. Addnl., the carboxyl-terminal 40 amino acids of VP3 are shown to  
be essential and sufficient for its interaction with VP1 in vitro. The

in

vitro assay system described here provides a method for identifying the  
domains involved in, and the mol. nature of, protein-protein

interactions,

which play an important role in such biol. phenomena as replication,  
transcription, translation, transport, ligand binding, and assembly.



L3 ANSWER 13 OF 18 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:3546 CAPLUS

DOCUMENT NUMBER: 118:3546

TITLE: Expression, **self-assembly**, and antigenicity of the Norwalk virus capsid protein

AUTHOR(S): Jiang, Xi; Wang, Min; Graham, David Y.; Estes, Mary K.

CORPORATE SOURCE: Div. Mol. Virol., Baylor Coll. Med., Houston, TX, 77030, USA

SOURCE: Journal of Virology (1992), 66(11), 6527-32

CODEN: JOVIAM; ISSN: 0022-538X

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Norwalk virus capsid protein was produced by expression of the 2nd and 3rd

open reading frames of the Norwalk virus genome, using a **cell-free** translation system and baculovirus recombinants. Anal. of the expressed products showed that the 2nd open reading frame encodes a protein with an apparent mol. wt. of 58,000 (58K protein) and that this protein self-assembles to form empty virus-like particles similar to native capsids in size and appearance. The antigenicity of these particles was demonstrated by immunopptn. and ELISA of paired serum samples from volunteers who developed illness following Norwalk virus challenge. These particles also induced high levels of Norwalk virus-specific serum antibody in lab. animals following parenteral inoculation. A minor 34K protein was also found in infected insect cells.

Amino acid sequence anal. of the N terminus of the 34K protein indicated that the 34K protein was a cleavage product of the 58K protein. The availability of large amts. of recombinant Norwalk virus particles will allow the development of rapid, sensitive, and reliable tests for the diagnosis of Norwalk virus infection as well as the implementation of structural studies.

L3 ANSWER 12 OF 18 CAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:735794 CAPLUS

DOCUMENT NUMBER: 126:17546

TITLE: **Self-assembly** of in  
vitro-translated human papillomavirus type 16 L1  
capsid protein into virus-like particles and

antigenic

reactivity of the protein

AUTHOR(S): Iyengar, Sujatha; Shah, Keerti V.; Kotloff, Karen L.;  
Ghim, Shin-Je; Viscidi, Raphael P.

CORPORATE SOURCE: Department Molecular Microbiology and Immunology,  
Johns Hopkins School Hygiene and Public Health,  
Baltimore, MD, 21205-2179, USA

SOURCE: Clinical and Diagnostic Laboratory Immunology (1996),  
3(6), 733-739

CODEN: CDIMEN; ISSN: 1071-412X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The human papillomavirus type 16 (HPV-16) L1 capsid protein is the major  
component of the HPV virion. We prepd. L1 protein of HPV-16 in a  
**cell-free** system. The L1 gene was cloned in an  
expression plasmid and transcribed and translated in vitro in a rabbit  
reticulocyte lysate. The expressed protein had the mol. mass (55 kDa)  
expected for the L1 protein, and it assembled into virus-like particles  
that closely resembled papillomavirus virions. The protein retained  
conformational epitopes, as evidenced by its reactivity with monoclonal  
antibodies which recognize only intact viral particles. In  
radioimmunopptn. assays with sera from college women grouped by their  
genital tract HPV DNA status, high reactivity was found in 68% of HPV-16  
DNA-pos. women, in 23% of women with other HPVs, and in 19% of HPV-neg.  
women. In comparison, none of the sera of children were reactive. The  
results of the radioimmunopptn. assays showed a significant correlation  
with results obtained with the same sera in an ELISA with virus-like  
particles produced in baculovirus (chi-square test for linear trend,  $P =$   
0.0023). Although the amts. of L1 protein obtained are small, the

ability

to produce virus-like particles by in vitro translation may be useful in  
the study of virus assembly, virus binding, and the immunol. response to  
HPV infection.

FILE 'BIOSIS' ENTERED AT 12:04:46 ON 31 OCT 2002  
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=> crystalization

L1 139 CRYSTALIZATION

=> protein and L1

L2 15 PROTEIN AND L1

=> virus and L2

L3 1 VIRUS AND L2

=> difficulty and L2

L4 0 DIFFICULTY AND L2

=> D L3 IBIB TI SO AU ABS

L3 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 1977:166932 BIOSIS  
DOCUMENT NUMBER: BA63:61796  
TITLE: FOLDING OF DNA MOLECULES IN CHROMATIN.  
AUTHOR(S): DAMASCHUN G  
SOURCE: ACTA BIOL MED GER, (1975 (RECD 1976)) 34 (11-12), NO  
PAGE.

CODEN: ABMGAJ. ISSN: 0001-5318.

FILE SEGMENT: BA; OLD

LANGUAGE: Unavailable

TI FOLDING OF DNA MOLECULES IN CHROMATIN.

SO ACTA BIOL MED GER, (1975 (RECD 1976)) 34 (11-12), NO PAGE.

CODEN: ABMGAJ. ISSN: 0001-5318.

AU DAMASCHUN G

AB A structural model for the folding of DNA in chromatin based on the X-ray diffraction patterns of deoxyribonucleoproteins (DNP) is presented. The DNA is oriented in the direction of DNP fibers and does not exhibit a superhelical structure. In the .nu.-bodies the DNA is folded 7 times on the envelope of a cylinder 10 nm in diameter. The height of the DNA hairpins is 9 nm-10 nm. The spacing between the refolded DNA segments is 3.6 nm. This supramolecular folding **crystalization** of the DNA is a general principle of organization and, through different types of morphological growth of the folding crystals, leads to the chromatin, to .psi.-DNA, to DNA monocrystals and to DNA packing in some phage heads.

binding of NCp7 to its nucleic acid targets. The mechanism of these activities is still debated but several clues are in favor of an intermediate aggregation of nucleic acids by NCp7. To check and characterize the nucleic acid aggregating properties of NCp7, we investigated the interaction of NCp7 with the model RNA homopolymer, polyA, by quasielastic light scattering and optical density measurements. The ordered growth of monodisperse large particles independently of the nucleic acid size and the almost complete covering of polyA by NCp7 strongly suggested an ordered aggregation mechanism. The aggregate kinetics of growth in the optimum protein concentration range (greater than or equal to 2  $\mu$  M) were governed by a so-called Ostwald ripening mechanism limited by transfer of NCp7-covered polyA complexes from small to large aggregates. The aggregation process was strongly dependent on both Na<sup>+</sup> and Mg<sup>2+</sup> concentrations, the optimum concentrations being in the physiological range. Similar conclusions held true when polyA was replaced by 16S+23S ribosomal RNA, suggesting that the NCp7 aggregating properties were only poorly dependent on the nucleic acid sequence and structure. Finally, as in the NCp7 annealing activities, the basic regions of NCp7, but not the zinc fingers, were found critical in nucleic acid aggregation. Taken together, our data indicate that NCp7 is a highly efficient nucleic acid aggregating agent and strengthen the hypothesis that aggregation may constitute a transient step in various NCp7 functions. (C) 1997 John Wiley & Sons, Inc.

L9 ANSWER 18 OF 44 MEDLINE DUPLICATE 7  
 ACCESSION NUMBER: 1998087828 MEDLINE  
 DOCUMENT NUMBER: 98087828 PubMed ID: 9426448  
 TITLE: Identification of a specific interaction between the coronavirus mouse hepatitis virus A59 nucleocapsid protein and packaging signal.  
 AUTHOR: Molenkamp R; Spaan W J  
 CORPORATE SOURCE: Department of Virology, Institute of Medical Microbiology, Leiden University, The Netherlands.  
 SOURCE: VIROLOGY, (1997 Dec 8) 239 (1) 78-86.  
 Journal code: 0110674. ISSN: 0042-6822.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199801  
 ENTRY DATE: Entered STN: 19980206  
 Last Updated on STN: 19980206  
 Entered Medline: 19980129

AB The coronavirus mouse hepatitis virus (MHV) is an enveloped positive stranded RNA virus. In infected cells MHV produces a 3' coterminal nested set of subgenomic messenger RNAs. Only the genomic RNA, however, is encapsidated by the nucleocapsid protein and incorporated in infectious MHV virions. It is believed that an RNA packaging signal (Ps), present only in the genomic RNA, is responsible for this selectivity. Earlier studies mapped this signal to a 69-nt stem-loop structure positioned in the 3' end of ORF1b. The selective encapsidation mechanism probably initiates by specific interaction of the packaging signal with the nucleocapsid protein. In this study we demonstrate the *in vitro* interaction of the MHV-A59 nucleocapsid protein with the packaging signal of MHV

using gel retardation and UV cross-linking assays. This interaction was observed not only with the nucleocapsid protein from infected cells but also with that from purified virions and from cells expressing a recombinant nucleocapsid protein. The specificity of the interaction was demonstrated by competition experiments with nonlabeled Ps containing RNAs, **tRNA**, and total cytoplasmic RNA. The results indicated that no virus specific modification of the N-protein or the presence of other viral proteins are required for this in **vitro** intervention. The assays described in this report provide us with a powerful tool for studying encapsidation (initiation) in more detail.

L9 ANSWER 19 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 97:44832 SCISEARCH

THE GENUINE ARTICLE: WA713

TITLE: NCp7 activates HIV-1(Lai) RNA dimerization by converting a transient loop-loop complex into a stable dimer

AUTHOR: Muriaux D; DeRocquigny H; Roques B P; Paoletti J (Reprint)

CORPORATE SOURCE: INST GUSTAVE ROUSSY, CNRS, URA 147, UNITE BIOCHIM, 39 RUE CAMILLE DESMOULINS, F-94805 VILLEJUIF, FRANCE (Reprint); INST GUSTAVE ROUSSY, CNRS, URA 147, UNITE BIOCHIM, F-94805 VILLEJUIF, FRANCE; UNIV PARIS 05, CNRS, URA D1500, INSERM, U266, DEPT PHARACOCHEM MOL & STRUCT, F-75006 PARIS, FRANCE

COUNTRY OF AUTHOR: FRANCE

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (27 DEC 1996) Vol. 271, No. 52, pp. 33686-33692.  
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.  
ISSN: 0021-9258.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 55

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Nucleocapsid **protein** 7 (NCp7), the human immunodeficiency **virus** type 1 (HTV-1) **nucleocapsid protein**, was shown to strongly potentiate the dimerization of the retroviral genomic RNA. This process involves the interaction of two retroviral RNA monomer subunits near their 5'-ends. A region located upstream from the splice donor site was recently identified as being responsible for the formation of dimeric HIV-1 RNA. This region appeared to be confined within a stem-loop structure, with an autocomplementary sequence in the loop. In an **in vitro** study of spontaneous dimer formation, we reported that the 77-402 RNA transcript forms two distinct dimers differing in their thermostability: D37 and D55. We identified D37 as a "kissing" complex structure, formed via a loop-loop interaction between the two monomers, and D55 as a double stranded structure involving all nucleotides of the stem loop via canonical base pairing. In this report, we have characterized the role of NCp7 in the HIV-1(Lai) RNA dimerization process by using **in vitro** dimerization assays with RNA transcripts of different lengths and dimer thermal dissociation. Our results show that the nucleocapsid protein NCp7 activates RNA dimerization very likely through interaction with the kissing complex and converts it into a stable

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dimer. Furthermore, this NCp7-promoted conversion only occurs if the 240-280 stem loop structure is present in HIV-1(Lai) RNA molecules and contains the autocomplementary G(257)CGCGC(262) sequence. This study suggests that, under physiological conditions, an NCp7-mediated RNA conformational change is involved in the maturation of the HIV-1 RNA dimer.

L9 ANSWER 20 OF 44 MEDLINE DUPLICATE 8  
ACCESSION NUMBER: 96323089 MEDLINE  
DOCUMENT NUMBER: 96323089 PubMed ID: 8709195  
TITLE: The zinc finger of **nucleocapsid protein** of Friend murine leukemia virus is critical for proviral DNA synthesis in vivo.  
AUTHOR: Yu Q; Darlix J L  
CORPORATE SOURCE: LaboRetro, Institut National de la Sante et de la Recherche Medicale U412, Ecole Normale Superieure de Lyon, France.  
SOURCE: JOURNAL OF VIROLOGY, (1996 Sep) 70 (9) 5791-8.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199609  
ENTRY DATE: Entered STN: 19960919  
Last Updated on STN: 19980206  
Entered Medline: 19960910

AB **Nucleocapsid protein** NCp10 of murine leukemia virus (MuLV) is encoded by the 3' domain of gag and contains a zinc finger of the form Cys-X2-Cys-X4-His-X4-Cys flanked by basic amino acids. In the course of virus assembly, NCp10 is necessary for core formation, and the zinc finger flanked by the basic residues is required for the packaging of the genomic RNA dimer. In **vitro**, NCp10 exhibits strong nucleic acid binding and annealing activities that appear to be critical for virus infectivity since NCp10 promotes dimerization of the viral RNA containing the E/DLS packaging-dimerization signal and annealing of replication primer **tRNA**(Pro) to the initiation site of reverse transcription (PBS). Recent in **vitro** studies have suggested that NCp10 may also play a role in proviral DNA synthesis. To investigate the function of NCp10 in proviral DNA synthesis in vivo, we developed a simple and convenient genetic packaging system consisting of two DNA constructs expressing the packaging components gag-pol and env of Friend MuLV and a Moloney MuLV-based lacZ vector with either the MuLV E+ or a rat VL30 E packaging signal. This system allowed us to examine the consequences of a set of mutations in NCp10 on a single round of recombinant virus replication. Most mutations in the N- or C-terminal domain of NCp10 do not significantly alter infectivity, while those in the zinc finger drastically impair infectivity. Analysis of the viral RNA content in virions showed that all mutations in the zinc finger decrease but do not abolish packaging of the recombinant genome. Interestingly enough, mutation of Y-28 to S (mutation Y28S) in the zinc finger results in RNA packaging at a level similar to that observed upon deletion of three prolines and three arginines in the C-terminal domain of NCp10 (mutant delta PR3). However, mutant Y28S is noninfectious while mutant delta PR3 is only threefold less

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infectious than the wild-type virus, which prompted us to examine the role of NCp10 protein in proviral DNA synthesis in vivo using these nucleocapsid mutants. PCR amplification was used to analyze viral DNA synthesized in newly infected cells, and results indicate that the Y28S zinc finger mutation impairs reverse transcription, thus suggesting that the nucleocapsid protein zinc finger plays a key role in proviral DNA synthesis in vivo.

L9 ANSWER 21 OF 44 MEDLINE DUPLICATE 9  
ACCESSION NUMBER: 96357022 MEDLINE  
DOCUMENT NUMBER: 96357022 PubMed ID: 8764006  
TITLE: Human immunodeficiency **virus** Type 1  
**nucleocapsid protein** (NCp7) directs  
specific initiation of minus-strand DNA synthesis  
primed by human **tRNA**(Lys3) in **vitro**  
: studies of viral RNA molecules mutated in regions  
that flank the primer binding site.  
AUTHOR: Li X; Quan Y; Arts E J; Li Z; Preston B D; de  
Rocquigny H; Roques B P; Darlix J L; Kleiman L;  
Parniak M A; Wainberg M A  
CORPORATE SOURCE: McGill University AIDS Center, Lady Davis  
Institute-Jewish General Hospital, Montreal, Quebec,  
Canada.  
SOURCE: JOURNAL OF VIROLOGY, (1996 Aug) 70 (8) 4996-5004.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199611  
ENTRY DATE: Entered STN: 19961219  
Last Updated on STN: 19961219  
Entered Medline: 19961107  
AB Retroviral reverse transcription starts near the 5' end of unspliced  
viral RNA at a sequence called the primer binding site (PBS), where  
the **tRNA** primer anneals to the RNA template for initiation  
of DNA synthesis. We have investigated the roles of NCp7 in  
annealing of primer **tRNA**(Lys3) to the PBS and in reverse  
transcriptase (RT) activity, using a cell-free reverse transcription  
reaction mixture consisting of various 5' viral RNA templates,  
natural primer **tRNA**(Lys3) or synthetic primer, human  
immunodeficiency **virus** type I (HIV-1) **nucleocapsid**  
**protein** (NCp7), and HIV-1 RT. In the presence of  
**tRNA**(Lys3), NCp7 was found to stimulate synthesis of  
minus-strand strong-stop DNA [(-)ssDNA], consistent with previous  
reports. However, specific DNA synthesis was observed only at a  
NCp7/RNA ratio similar to that predicted to be present in virions.  
Moreover, at these concentrations, NCp7 inhibited the synthesis of  
nonspecific reverse-transcribed DNA products, which are initiated  
because of self-priming by RNA templates. In contrast to results  
obtained with **tRNA**(Lys3) as primer, NCp7 inhibited the  
synthesis of (-)ssDNA products primed by an 18-nucleotide (nt)  
ribonucleotide (rPR), complementary to the PBS, even though rPR can  
initiate synthesis of such material in the absence of preannealing  
with NCp7. Primer placement band shift assays showed that NCp7 was  
necessary for efficient formation of the **tRNA**-RNA complex.  
In contrast, NCp7 was found to prevent formation of the rPR-RNA  
complex. Since NCp7 appears to exert opposite effects (annealing

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versus dissociation) on **tRNA**(Lys3) and rPR substrates, the non-PBS binding regions of the **tRNA**(Lys3) molecule may play a role in the annealing of **tRNA** to the template. We also investigated the roles of an A-rich loop upstream of the PBS, a 7-nt region immediately downstream of the PBS, and a 54-nt deletion further downstream of the PBS in interactions with **tRNA**(Lys3). We found that deletions in the 54-nt region that may prevent formation of the U5-leader stem prevented **tRNA**(Lys3) placement and priming, while deletions in the A-rich loop or the 7-nt sequence had relatively minor effects in this regard.

L9 ANSWER 22 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
ACCESSION NUMBER: 96:73111 SCISEARCH  
THE GENUINE ARTICLE: TP526  
TITLE: MUTATIONS OF BASIC-AMINO-ACIDS OF NCP7 OF  
HUMAN-IMMUNODEFICIENCY-VIRUS TYPE-1 AFFECT  
RNA-BINDING IN-VITRO  
AUTHOR: SCHMALZBAUER E; STRACK B; DANNULL J; GUEHMANN S;  
MOELLING K (Reprint)  
CORPORATE SOURCE: UNIV ZURICH, INST MED VIROL, GLORIASTR 30, CH-8028  
ZURICH, SWITZERLAND (Reprint); UNIV ZURICH, INST MED  
VIROL, CH-8028 ZURICH, SWITZERLAND  
COUNTRY OF AUTHOR: SWITZERLAND  
SOURCE: JOURNAL OF VIROLOGY, (FEB 1996) Vol. 70, No. 2, pp.  
771-777.  
ISSN: 0022-538X.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 42

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **nucleocapsid** (NC) **protein** of human immunodeficiency **virus** type 1 is required for packaging of viral RNA and for virion assembly. It contains two clusters of basic amino acids, consisting of five and four amino acid residues, flanking the first of its two zinc fingers. These amino acid residues have been mutagenized to neutral ones individually, as well as in various combinations, by site-directed mutagenesis. Wild-type NCp7 and the mutant proteins were expressed as recombinant proteins in *Escherichia coli*, with six histidines as tags at their amino termini in order to allow efficient purification. The purified proteins were analyzed for RNA binding in **vitro** with human immunodeficiency virus type 1 5' leader RNA transcribed in **vitro**. Assays comprised Northwestern blots at various salt concentrations and filter binding tests which allowed determination of the dissociation constants of the various mutants. The results indicated that mutations of the amino acid R-7 and of R-32 and K-33 were more critical for RNA binding than other mutations. Mutation of the other amino acid residues reduced the binding affinity in proportion to the number of mutations. Mutation of seven of the nine basic amino acid residues reduced the binding of RNA by 50- to 90-fold.

L9 ANSWER 23 OF 44 MEDLINE DUPLICATE 10  
ACCESSION NUMBER: 96215651 MEDLINE  
DOCUMENT NUMBER: 96215651 PubMed ID: 8645104  
TITLE: RNA binding properties of core protein of the  
flavivirus Kunjin.



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AUTHOR: Khromykh A A; Westaway E G  
CORPORATE SOURCE: Sir Albert Sakzewski Virus Research Centre, Royal  
Children's Hospital, Brisbane, Australia.  
SOURCE: ARCHIVES OF VIROLOGY, (1996) 141 (3-4) 685-99.  
Journal code: 7506870. ISSN: 0304-8608.  
PUB. COUNTRY: Austria  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199607  
ENTRY DATE: Entered STN: 19960726  
Last Updated on STN: 19980206  
Entered Medline: 19960715

AB Kunjin virus (KUN) C is a typical flavivirus core protein which is truncated in vivo to a mature form of 105 residues enriched in lysine and arginine. In order to study the possible association of KUN C with RNA in *vitro*, we prepared several recombinant C proteins with specific deletions, each fused at the amino-terminus to glutathione-S-transferase (GST) and expressed in *E. coli*. They were reacted with KUN RNA probes transcribed in *vitro* from cDNA representing the 5' untranslated region (5' UTR, 93 to 96 nucleotides), the 3' UTR (624 nucleotides), and the 5' UTR plus most of the C coding region (5' core, 440 nucleotides). Fusion protein C107 (incorporating mature C) bound strongly to all KUN RNA probes with apparent specificity, being completely resistant to inhibition by 800 mM NaCl, and to competition by a large excess of **tRNA**. In reactions with labelled KUN RNA probes putative binding sites were identified in the isolated amino-terminal (32 residues) and carboxy-terminal (26 residues) basic amino acid domains; this binding was strongly competed by unlabelled KUN UTR probes but weakly or not at all by **tRNA**. These small domains probably acted co-operatively in binding of mature C to KUN RNA probes. The KUN RNA-core protein binding reactions are similar to those reported with other **viral** coat or **capsid proteins** and **viral** RNAs.

L9 ANSWER 24 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 96:894836 SCISEARCH

THE GENUINE ARTICLE: VV421

TITLE: A specific RNA structural motif mediates high affinity binding by the HIV-1 nucleocapsid protein (NCp7)

AUTHOR: Allen P (Reprint); Collins B; Brown D; Hostomsky Z; Gold L

CORPORATE SOURCE: UNIV COLORADO, DEPT MOL CELLULAR & DEV BIOL, CAMPUS BOX 347, BOULDER, CO 80309 (Reprint); AGOURON PHARMACUET INC, SAN DIEGO, CA 92121

COUNTRY OF AUTHOR: USA

SOURCE: VIROLOGY, (15 NOV 1996) Vol. 225, No. 2, pp. 306-315

Publisher: ACADEMIC PRESS INC JNL-COMP SUBSCRIPTIONS  
525 B ST, STE 1900, SAN DIEGO, CA 92101-4495.  
ISSN: 0042-6822.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 45

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

Searcher : Shears 308-4994

AB Current research indicates that the **nucleocapsid protein** (NCp7) of human immunodeficiency **virus** type 1 (HIV-1) interacts with a variety of RNA substrates during the progression of the viral life cycle. The RNA features specifically recognized by the protein, however, have yet to be identified. SELEX was used to generate a set of RNAs whose affinities for nucleocapsid were on the order of  $2 \times 10^{-9}$  M. Comparative analysis revealed that each RNA contains a highly conserved fourteen nucleotide sequence-block. Computer modeling and structure probing experiments indicate that the RNA ligands use the consensus sequence to fold into hairpins with an identical asymmetric bulge. The presence of the nucleocapsid protein protects the asymmetric bulge from ribonuclease attack, suggesting that it is the key element in protein recognition. A search for similar structural motifs within the HIV genome reveals several potential interaction sites for the nucleocapsid protein. (C) 1996 Academic Press, Inc.

L9 ANSWER 25 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
DUPLICATE 11

ACCESSION NUMBER: 1996:228892 BIOSIS  
DOCUMENT NUMBER: PREV199698785021  
TITLE: Mutations at the capsid-nucleocapsid cleavage site of gag polyprotein of Moloney murine leukemia virus abolish virus infectivity.  
AUTHOR(S): Housset, Valerie; Darlix, Jean-Luc (1)  
CORPORATE SOURCE: (1) LaboRetro, Unite Virol. Hum., INSERM-ENS U412, Ec. Norm. Super. Lyon, 46 allée d'Italie, 69634 Lyon Cedex France  
SOURCE: Comptes Rendus de l'Academie des Sciences Serie III Sciences de la Vie, (1996) Vol. 319, No. 2, pp. 81-89.  
ISSN: 0764-4469.  
DOCUMENT TYPE: Article  
LANGUAGE: English; French  
SUMMARY LANGUAGE: English; French

AB Capsid protein CAP30 and nucleocapsid protein NCp10 of Moloney murine leukemia virus (MoMuLV) are the 2 major proteic components of the virion core and are generated by processing of the gag polyprotein precursor, Pr65-gag, by the viral protease. In the virion core, several hundred NCp10 molecules are bound to the genomic RNA dimer forming the **nucleocapsid** structure. In the course of **virus** assembly, NC **protein**, as the mature NCp10 and/or as the gag precursor, appears to direct genomic RNA packaging. In **vitro**, NCp10 has nucleic acid binding and annealing activities and promotes viral RNA dimerization and the annealing of replication primer **tRNA**-Pro to the primer binding site (PBS) which is necessary for the initiation of reverse transcription. To investigate whether maturation of NCp10 is required for virus formation, we substituted charged residues for the hydrophobic amino acids at the capsid-nucleocapsid protein cleavage site in order to prevent maturation of NCp10. Here we report that these mutations abolished maturation of capsid protein CAP30 and NCp 10 by the viral protease in **vitro**. When these mutations were introduced into an infectious MoMuL V molecular clone, Pr65-gag precursor was synthesized in transfected cells but virion production was strongly diminished and mutant viruses were not infectious. These results suggest that maturation of NCp10 is required for optimal virion release and production of infectious

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virus.

L9 ANSWER 26 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.DUPLICATE  
12

ACCESSION NUMBER: 95102944 EMBASE

DOCUMENT NUMBER: 1995102944

TITLE: Synthesis and conformational studies of a cyclic analog of the proximal zinc finger of HIV-1 NCP7 for antibody generation.

AUTHOR: Dong C.Z.; Jullian N.; Yang Y.S.; De Rocquigny H.; Fournie-Zaluski M.C.; Roques B.P.

CORPORATE SOURCE: Dept. de Pharmacochim.Molec./Struct., CNRS URA D 150, Faculte de Pharmacie, 4 Avenue de

SOURCE: l'Observatoire, 75270 Paris Cedex 06, France  
Journal of the American Chemical Society, (1995)  
117/10 (2726-2731).

ISSN: 0002-7863 CODEN: JACSAT

COUNTRY: United States

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 004 Microbiology  
029 Clinical Biochemistry  
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus** type 1 (HIV-1) contains two zinc finger domains (CCHC boxes), which have been shown to be very important in the viral replication cycle. However, these domains are not involved directly in either in **vitro** RNA dimerization or **tRNA**(Lys,3) annealing. For a more detailed understanding of the role of the zinc fingers in the different functions of NCp7, antibodies directed against these domains would be very useful. For this purpose, a cyclic peptide analog of the proximal zinc finger (13-30)NCp7 has been synthesized in solid phase using a strategy of combined Fmoc and Boc chemistry. On the basis of the 3D structural data of NCp7, the Asn17 and Ala30 have been changed to Glu17 and Lys30 and a cyclization carried out between their side chains. The structures of the cyclic and native peptides complexed with Co<sup>2+</sup> and Zn<sup>2+</sup> were studied by visible and 2D 1H NMR spectroscopy, respectively. The nuclear Overhauser effects obtained were applied as constraints to determine the solution structures using DIANA software followed by AMBER energy refinement. The results show that the cyclic peptide retains the highly folded structure of the native peptide and exhibits an enhanced affinity for metallic ions. These are favorable parameters for the generation of antibodies against the zinc fingers in NCp7.

L9 ANSWER 27 OF 44 MEDLINE DUPLICATE 13

ACCESSION NUMBER: 95130539 MEDLINE

DOCUMENT NUMBER: 95130539 PubMed ID: 7829498

TITLE: Binding of the HIV-1 nucleocapsid protein to the primer **tRNA**(3Lys), in **vitro**, is essentially not specific.

AUTHOR: Mely Y; de Rocquigny H; Sorinas-Jimeno M; Keith G; Roques B P; Marquet R; Gerard D

CORPORATE SOURCE: Laboratoire de Biophysique, URA 491 du CNRS, Universite Louis Pasteur de Strasbourg I, Faculte de Pharmacie, Illkirch, France.

Searcher : Shears 308-4994

09/713687

SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1995 Jan 27) 270  
(4) 1650-6.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199502  
ENTRY DATE: Entered STN: 19950307  
Last Updated on STN: 19990129  
Entered Medline: 19950222

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus**, type 1, is a key component in the viral life cycle. Since, the first common step of all reported NCp7 activities corresponds to a nucleic acid-binding step, the NCp7 binding parameters to the natural primer **tRNA**(3Lys) were investigated. Using NCp7 intrinsic fluorescence, we found that (i) in 0.1 M NaCl, NCp7 bound noncooperatively to **tRNA**(3Lys) with a  $K_{obs} = 3.2 \times 10^6$  M<sup>-1</sup> association constant and a  $n = 6$  binding site size, (ii) four ionic interactions were formed in the NCp7.**tRNA**(3Lys) complex, and (iii) nonelectrostatic factors provided about 60% of the binding energy. These binding parameters were not significantly altered when the natural **tRNA**(3Lys) was replaced by either an *in vitro* synthetic **tRNA**(3Lys) transcript, the heterologous yeast **tRNA**(Phe) or the structurally unrelated 5 S RNA from *Escherichia coli*. Moreover, the environment of the intrinsic fluorescent reporters (Trp37 and Trp61) was similar in the various complexes. Finally, experiments performed at low protein concentration provide no evidence of high affinity binding sites. Taken together, our data strongly suggested an essentially nonspecific binding of NCp7 to **tRNA**(3Lys) and thus did not seem to support a direct role of NCp7, *per se*, in the selection of **tRNA**(3Lys) from the pool of cellular **tRNAs**.

L9 ANSWER 28 OF 44 MEDLINE DUPLICATE 14  
ACCESSION NUMBER: 95001879 MEDLINE  
DOCUMENT NUMBER: 95001879 PubMed ID: 7918387  
TITLE: 1H NMR structure and biological studies of the His23-->Cys mutant nucleocapsid protein of HIV-1 indicate that the conformation of the first zinc finger is critical for virus infectivity.  
AUTHOR: Demene H; Dong C Z; Ottmann M; Rouyez M C; Jullian N; Morellet N; Mely Y; Darlix J L; Fournie-Zaluski M C; Saragosti S; +  
CORPORATE SOURCE: Departement de Pharmacochimie Moleculaire et Structurale, U266 INSERM-URA D1500 CNRS, Faculte de Pharmacie, Universite Rene Descartes, Paris, France.  
SOURCE: BIOCHEMISTRY, (1994 Oct 4) 33 (39) 11707-16.  
Journal code: 0370623. ISSN: 0006-2960.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199411  
ENTRY DATE: Entered STN: 19941222  
Last Updated on STN: 20000303  
Entered Medline: 19941108

Searcher : Shears 308-4994

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus** type 1 (HIV-1), which has key functions in the virus life cycle, possesses two zinc fingers of the CX2CX4HX4C type characterized by three successive loops containing a tetrahedrally coordinated zinc atom. The replacement of any cysteine by a serine in either finger has been shown to result in the production of noninfectious viruses, probably by impairing the biological functions of NCp7. In order to more precisely elucidate the structural role of the zinc finger motif, His23 was replaced by Cys in the proximal finger of the peptide (13-64)NCp7 which retains NCp7 activities in **vitro**. The peptide Cys23(13-64)NCp7 was synthesized by solid phase and studied by 2D 1H NMR and molecular modeling. The His to Cys modification causes important structural modifications of the N-terminal zinc finger which impair the spatial proximity of the two zinc fingers as shown by the disappearance of several interresidue NOEs. The side chains of Val13, Lys14, Phe16, Thr24, Ala25, Trp37, Gln45, and Met46, which are thought to be involved in nucleic acid recognition, are no longer found clustered in the Cys23(13-64)NCp7 mutant as they are in the wild-type NCp7 structure. In **vitro**, Cys23(13-64)NCp7 is unable to tightly interact with the viral RNA or replication primer **tRNA** (Lys,3). The Cys23(NCp7) mutation was introduced into an infectious HIV-1 molecular clone, and virions produced upon DNA transfection into cells were analyzed for their viral protein and RNA compositions as well as for their infectivity. Results show that, while the Cys23(NCp7) mutation does not impair virion production, viruses contain a low amount of degraded viral RNA and are not infectious. These findings suggest that a bona fide conformation of the HIV-1 NCp7 is critical for the packaging of viral RNA, its stability in virions, and virus infectivity.

L9 ANSWER 29 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
 ACCESSION NUMBER: 94:235001 SCISEARCH  
 THE GENUINE ARTICLE: NF928  
 TITLE: SPECIFIC BINDING OF HIV-1 NUCLEOCAPSID PROTEIN TO  
 PSI-RNA IN-VITRO REQUIRES N-TERMINAL  
 ZINC-FINGER AND FLANKING BASIC-AMINO-ACID RESIDUES  
 AUTHOR: DANNULL J; SUROVOY A; JUNG G; MOELLING K (Reprint)  
 CORPORATE SOURCE: UNIV ZURICH, INST MED VIROL, CH-8028 ZURICH,  
 SWITZERLAND (Reprint); MAX PLANCK INST MOLEC GENET,  
 SCHUSTER ABT, D-14195 BERLIN, GERMANY; UNIV  
 TUBINGEN, INST ORGAN CHEM, D-72076 TUBINGEN, GERMANY  
 COUNTRY OF AUTHOR: SWITZERLAND; GERMANY  
 SOURCE: EMBO JOURNAL, (01 APR 1994) Vol. 13, No. 7, pp.  
 1525-1533.  
 ISSN: 0261-4189.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 54

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **nucleocapsid** (NC) **protein** of human immunodeficiency **virus** HIV-1 (NCp7) is responsible for packaging the viral RNA by recognizing a packaging site (PSI) on the viral RNA genome. NCp7 is a molecule of 55 amino acids containing two zinc fingers, with only the first one being highly conserved among retroviruses. The first zinc finger is flanked by two basic amino acid clusters. Here we demonstrate that chemically synthesized

NCp7 specifically binds to viral RNA containing the PSI using competitive filter binding assays. Deletion of the PSI from the RNA abrogates this effect. The 35 N-terminal amino acids of NCp7, comprising the first zinc finger, are sufficient for specific RNA binding. Chemically synthesized mutants of the first zinc finger demonstrate that the amino acid residues C-C-C/H-C/H are required for specific RNA binding and zinc coordination. Amino acid residues F16 and T24, but not K20, E21 and G22, located within this zinc finger, are essential for specific RNA binding as well. The second zinc finger cannot replace the first one. Furthermore, mutations in the basic amino acid residues flanking the first zinc finger demonstrate that R3, 7, 10, 29 and 32 but not K11, 14, 33 and 34 are also essential for specific binding. Specific binding to viral RNA is also observed with recombinant NCp15 and Pr55Gag. The results demonstrate for the first time specific interaction of a retroviral NC protein with its PSI RNA *in vitro*.

L9 ANSWER 30 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
 ACCESSION NUMBER: 94:145398 SCISEARCH  
 THE GENUINE ARTICLE: MX771  
 TITLE: TRANSACTIVATION OF THE MINUS-STRAND DNA TRANSFER BY NUCLEOCAPSID PROTEIN DURING REVERSE TRANSCRIPTION OF THE RETROVIRAL GENOME  
 AUTHOR: ALLAIN B; LAPADATTAPOLSKY M; BERLIOZ C; DARLIX J L (Reprint)  
 CORPORATE SOURCE: ECOLE NORMALE SUPER LYON, INSERM, LABORETRO, 46 ALLEE ITALIE, F-69364 LYON, FRANCE (Reprint); ECOLE NORMALE SUPER LYON, INSERM, LABORETRO, F-69364 LYON, FRANCE  
 COUNTRY OF AUTHOR: FRANCE  
 SOURCE: EMBO JOURNAL, (15 FEB 1994) Vol. 13, No. 4, pp. 973-981.  
 ISSN: 0261-4189.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 30

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Two DNA strand transfers are required during reverse transcription of the RNA genome of retroviruses to complete provirus synthesis. To understand more about the first strand transfer reaction, that of the minus-strand DNA from the 5' to the 3' end of the retroviral genome, we devised an *in vitro* system mimicking the Moloney murine leukemia virus reverse transcription process. Two RNAs corresponding to the 5' and 3' regions of the genome were used to perform reverse transcription assays. The role of the nucleocapsid protein NCp10, which is tightly bound to the genome in the virus, was investigated in this system as well as the requirement of the 5' and 3' terminal repeats (R sequences) and the poly(A) tail. The results show that NCp10 drastically enhances the strand transfer reaction and that interactions between reverse transcriptase, **nucleocapsid protein** and **viral RNA** may be important. Both R sequences are required for an efficient and accurate DNA strand transfer and the poly(A) tail facilitates this reaction. Furthermore, it is probable that both intra- and intermolecular DNA strand transfers occur when the 5' and 3' ends of the genome are present on the same molecule.

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L9 ANSWER 31 OF 44 MEDLINE DUPLICATE 15  
ACCESSION NUMBER: 93281729 MEDLINE  
DOCUMENT NUMBER: 93281729 PubMed ID: 8506369  
TITLE: Identification of a binding site for the human  
immunodeficiency **virus** type 1  
**nucleocapsid protein**.  
AUTHOR: Sakaguchi K; Zambrano N; Baldwin E T; Shapiro B A;  
Erickson J W; Omichinski J G; Clore G M; Gronenborn A  
M; Appella E  
CORPORATE SOURCE: Laboratory of Cell Biology, National Cancer  
Institute, Bethesda, MD 20892.  
CONTRACT NUMBER: N01-CO-74102 (NCI)  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF  
THE UNITED STATES OF AMERICA, (1993 Jun 1) 90 (11)  
5219-23.  
Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199307  
ENTRY DATE: Entered STN: 19930716  
Last Updated on STN: 19980206  
Entered Medline: 19930707  
AB The nucleocapsid (NC) protein NCp7 of human immunodeficiency virus  
type 1 (HIV-1) is important for encapsidation of the virus genome,  
RNA dimerization, and primer **tRNA** annealing in  
**vitro**. Here we present evidence from gel mobility-shift  
experiments indicating that NCp7 binds specifically to an RNA  
sequence. Two complexes were identified in native gels. The more  
slowly migrating complex contained two RNA molecules and one  
peptide, while the more rapidly migrating one is composed of one RNA  
and one peptide. Further, mutational analysis of the RNA shows that  
the predicted stem and loop structure of stem-loop 1 plays a  
critical role. Our results show that NCp7 binds to a unique RNA  
structure within the psi region; in addition, this structure is  
necessary for RNA dimerization. We propose that NCp7 binds to the  
RNA via a direct interaction of one zinc-binding motif to stem-loop  
1 followed by binding of the other zinc-binding motif to stem-loop  
1, stem-loop 2, or the linker region of the second RNA molecule,  
forming a bridge between the two RNAs.

L9 ANSWER 32 OF 44 MEDLINE DUPLICATE 16  
ACCESSION NUMBER: 93233217 MEDLINE  
DOCUMENT NUMBER: 93233217 PubMed ID: 8474159  
TITLE: Basic amino acids flanking the zinc finger of Moloney  
murine leukemia **virus nucleocapsid**  
**protein** NCp10 are critical for **virus**  
infectivity.  
AUTHOR: Housset V; De Rocquigny H; Roques B P; Darlix J L  
CORPORATE SOURCE: LaboRetro Institut National de la Sante et de la  
Recherche Medicale, Ecole Normale Superieure de Lyon,  
France.  
SOURCE: JOURNAL OF VIROLOGY, (1993 May) 67 (5) 2537-45.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English

Searcher : Shears 308-4994

09/713687

FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199305  
ENTRY DATE: Entered STN: 19930604  
Last Updated on STN: 19970203  
Entered Medline: 19930517

AB Nucleocapsid (NC) protein NCp10 of Moloney murine leukemia virus is encoded by the 3' domain of gag and contains a zinc finger surrounded by basic amino acids. During virion assembly, NC protein is necessary for core formation and the NC zinc finger is required for the packaging of the genomic RNA dimer. In *vitro* NCp10 has RNA-binding and -annealing activities critical for virus infectivity, since NCp10 promotes dimerization of viral RNA containing the Psi packaging element and annealing of replication primer *tRNA*(Pro) to the initiation site of reverse transcription (primer-binding site). To investigate the role of the basic amino acids flanking the NCp10 zinc finger, neutral residues were substituted for the basic amino acids and the effects of these mutations in vivo on virus assembly and infectivity and in *vitro* on the RNA-annealing activity of NCp10 were analyzed. Here we report that the substitution of 1 or 2 neutral amino acids for the basic residues did not impair the production of mature virions but that infectivity was either moderately or strongly attenuated. When more than 2 basic residues were replaced by neutral amino acids, viruses were poorly infectious because of a severe defect in genomic RNA dimer packaging and initiation of reverse transcription. In *vitro* NCp10-derived peptides with similar mutations were chemically synthesized and were found to be either fully or partially active or completely inactive. These data indicate that the basic residues flanking the zinc finger of NCp10 are required for the production of infectious Moloney murine leukemia virus virions.

L9 ANSWER 33 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 93:171068 SCISEARCH

THE GENUINE ARTICLE: KR189

TITLE: INTERACTIONS BETWEEN HIV-1 NUCLEOCAPSID  
PROTEIN AND VIRAL-DNA MAY HAVE  
IMPORTANT FUNCTIONS IN THE VIRAL LIFE-CYCLE

AUTHOR: LAPADATTAPOLSKY M; DEROCQUIGNY H; VANGENT D; ROQUES  
B; PLASTERK R; DARLIX J L (Reprint)

CORPORATE SOURCE: ECOLE NORMALE SUPER LYON, LABORETRO INSERM, 46 ALLEE  
DITALIE, F-69364 LYON, FRANCE; UNIV PARIS 05, UNITE  
PHARMACOCHEM MOLEC & STRUCT, CNRS, URA D1500,  
INSERM, U266, F-75270 PARIS 06, FRANCE; NETHERLANDS  
CANC INST, DIV MOLEC BIOL, 1066 CX AMSTERDAM,  
NETHERLANDS

COUNTRY OF AUTHOR: FRANCE; NETHERLANDS

SOURCE: NUCLEIC ACIDS RESEARCH, (25 FEB 1993) Vol. 21, No.  
4, pp. 831-839.  
ISSN: 0305-1048.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: ENGLISH

REFERENCE COUNT: 36

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB In the virion core of retroviruses, the genomic RNA is tightly associated with nucleocapsid (NC) protein molecules, forming the nucleocapsid structure. NC protein, a highly basic protein with two



zinc fingers, is indispensable for RNA dimerization, encapsidation and the initiation of reverse transcription in avian, murine and human retroviruses. Here we show that NC protein of HIV-1 (NCp7) and NCp7 mutants bind to DNA fragments representing proviral DNA sequences, forming stable complexes. NCp7 and NCp7 mutants form high molecular weight complexes with large DNA fragments and show cooperativity in binding to the DNAs. It appears that the conserved basic residues, and not the zinc fingers, are important for complex formation. In addition, NCp7 and several NCp7 mutants protect DNAs from nuclease digestion while the DNA ends appear to be poorly protected. NCp7 has been found to bind to strong stop cDNA, the initial product of reverse transcription, and to promote the annealing of this cDNA to HIV-1 RNA corresponding to the 3' end of the genome. In addition, NCp7 slightly stimulates an *in vitro* IN cleavage assay. These results indicate that the interactions between NCp7 and proviral DNA may be important during provirus synthesis and/or prior to integration.

L9 ANSWER 34 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
 ACCESSION NUMBER: 93:592908 SCISEARCH  
 THE GENUINE ARTICLE: LY812  
 TITLE: TRANSACTIVATION OF THE 5' TO 3' **VIRAL**-DNA  
 STRAND TRANSFER BY **NUCLEOCAPSID**  
**PROTEIN** DURING REVERSE TRANSCRIPTION OF  
 HIV-1 RNA  
 AUTHOR: DARLIX J L (Reprint); VINCENT A; GABUS C;  
 DEROCQUIGNY H; ROQUES B  
 CORPORATE SOURCE: ECOLE NORMALE SUPER LYON, INSERM, LABORETRO, 46  
 ALLEE ITALIE, F-69364 LYON 07, FRANCE (Reprint);  
 UNIV PARIS 05, FAC PHARM, INSERM, U266, F-75270  
 PARIS, FRANCE  
 COUNTRY OF AUTHOR: FRANCE  
 SOURCE: COMPTES RENDUS DE L ACADEMIE DES SCIENCES SERIE  
 III-SCIENCES DE LA VIE-LIFE SCIENCES, (AUG 1993)  
 Vol. 316, No. 8, pp. 763-771.  
 ISSN: 0764-4469.  
 DOCUMENT TYPE: Article; Journal  
 FILE SEGMENT: LIFE; AGRI  
 LANGUAGE: ENGLISH  
 REFERENCE COUNT: 33

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Two DNA strand transfer reactions take place during reverse transcription of the retroviral genome. The first transfer, that of the minus-strand strong stop DNA from the 5' end of the viral RNA to the 3' end, has been studied in *vitro* with two RNAs mimicking the 5' and 3' regions of the HIV1 genome and with nucleocapsid protein, NCp7, and reverse transcriptase. The results show that NCp7 strongly activates the 5' to 3' DNA strand transfer during reverse transcription while a basic peptide resembling NCp7 is inactive. Activation of the first transfer by several NCp7 derived peptides and the influence of the terminal redundancies (R) present at the 5' and 3' ends of HIV1 RNA were also examined. The first transfer is optimal in the presence of intact NCp7 and necessitates R on both the 5' and 3' RNAs. Sequencing of full length viral DNA products reveals approximately 40 % misincorporations at the first nucleotide beyond the transfer point. If such base misincorporations occur during proviral DNA synthesis with possible homologous recombinations it may well contribute to the high level

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of genetic variability of HIV.

L9 ANSWER 35 OF 44 MEDLINE DUPLICATE 17  
ACCESSION NUMBER: 92335320 MEDLINE  
DOCUMENT NUMBER: 92335320 PubMed ID: 1631144  
TITLE: Viral RNA annealing activities of human  
immunodeficiency virus type 1  
nucleocapsid protein require only  
peptide domains outside the zinc fingers.  
AUTHOR: De Rocquigny H; Gabus C; Vincent A; Fournie-Zaluski M  
C; Roques B; Darlix J L  
CORPORATE SOURCE: Departement de Chimie Organique, U266 Institut  
National de la Sante et de la Recherche Medicale  
Unite Associee 498, Universite Rene Descartes, Paris,  
France.  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF  
THE UNITED STATES OF AMERICA, (1992 Jul 15) 89 (14)  
6472-6.  
Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199208  
ENTRY DATE: Entered STN: 19920904  
Last Updated on STN: 19980206  
Entered Medline: 19920818

AB The nucleocapsid (NC) of human immunodeficiency virus type 1 consists of a large number of NC protein molecules, probably wrapping the dimeric RNA genome within the virion inner core. NC protein is a gag-encoded product that contains two zinc fingers flanked by basic residues. In human immunodeficiency virus type 1 virions, NCp15 is ultimately processed into NCp7 and p6 proteins. During virion assembly the retroviral NC protein is necessary for core formation and genomic RNA encapsidation, which are essential for virus infectivity. In *vitro* NCp15 activates viral RNA dimerization, a process most probably linked in vivo to genomic RNA packaging, and replication primer tRNA(Lys,3) annealing to the initiation site of reverse transcription. To characterize the domains of human immunodeficiency virus type 1 NC protein necessary for its various functions, the 72-amino acid NCp7 and several derived peptides were synthesized in a pure form. We show here that synthetic NCp7 with or without the two zinc fingers has the RNA annealing activities of NCp15. Further deletions of the N-terminal 12 and C-terminal 8 amino acids, leading to a 27-residue peptide lacking the finger domains, have little or no effect on NC protein activity in *vitro*. However deletion of short sequences containing basic residues flanking the first finger leads to a complete loss of NC protein activity. It is proposed that the basic residues and the zinc fingers cooperate to select and package the genomic RNA in vivo. Inhibition of the viral RNA binding and annealing activities associated with the basic residues flanking the first zinc finger of NC protein could therefore be used as a model for the design of antiviral agents.

L9 ANSWER 36 OF 44 MEDLINE DUPLICATE 18  
ACCESSION NUMBER: 93077033 MEDLINE  
DOCUMENT NUMBER: 93077033 PubMed ID: 1280240

Searcher : Shears 308-4994

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TITLE: Recombinant HIV-1 nucleocapsid protein p15 produced as a fusion protein with glutathione S-transferase in Escherichia coli mediates dimerization and enhances reverse transcription of retroviral RNA.  
AUTHOR: Weiss S; Konig B; Morikawa Y; Jones I  
CORPORATE SOURCE: Boehringer Mannheim GmbH, Research Center Penzberg, Germany.  
SOURCE: GENE, (1992 Nov 16) 121 (2) 203-12.  
Journal code: 7706761. ISSN: 0378-1119.  
PUB. COUNTRY: Netherlands  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199212  
ENTRY DATE: Entered STN: 19930129  
Last Updated on STN: 19990129  
Entered Medline: 19921230

AB Human immunodeficiency virus 1 (HIV-1) **nucleocapsid protein** p15 was produced as a fusion protein with glutathione S-transferase (GST) in Escherichia coli. Rapid purification of GST::p15 in an active form by one-step glutathione-agarose chromatography was accomplished in the presence of an antioxidant. Recombinant p15 fused to GST was shown to stimulate the dimerization of viral RNA. HIV-1 reverse transcriptase-catalyzed in **vitro** synthesis of minus-strand cDNA from synthetic human **tRNA**(Lys3UUU) and natural bovine **tRNA**(Lys3SUU) primer molecules was enhanced by GST::p15. GST produced in E.coli revealed no effect with respect to RNA dimerization and cDNA synthesis, demonstrating that both activities reside in the p15 portion of the fusion protein.

L9 ANSWER 37 OF 44 MEDLINE DUPLICATE 19  
ACCESSION NUMBER: 91305094 MEDLINE  
DOCUMENT NUMBER: 91305094 PubMed ID: 1906602  
TITLE: **Viral** RNA annealing activities of the **nucleocapsid protein** of Moloney murine leukemia virus are zinc independent.  
AUTHOR: Prats A C; Housset V; de Billy G; Cornille F; Prats H; Roques B; Darlix J L  
CORPORATE SOURCE: Centre de Recherche de Biochimie et Genetique Cellulaires du CNRS, Toulouse, France.  
SOURCE: NUCLEIC ACIDS RESEARCH, (1991 Jul 11) 19 (13) 3533-41.  
Journal code: 0411011. ISSN: 0305-1048.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 199108  
ENTRY DATE: Entered STN: 19910908  
Last Updated on STN: 20000303  
Entered Medline: 19910820

AB The zinc fingers of retroviral gag nucleocapsid proteins (NC) are required for the specific packaging of the dimeric RNA genome into virions. In **vitro**, NC proteins activate both dimerization of viral RNA and annealing of the replication primer **tRNA** onto viral RNA, two reactions necessary for the production of infectious virions. In this study the role of the zinc finger of

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Moloney murine leukemia virus (MoMuLV) NCp10 in RNA binding and annealing activities was investigated through modification or replacement of residues involved in zinc coordination. These alterations did not affect the ability of NCp10 to bind RNA and promote RNA annealing in *vitro*, despite a complete loss of zinc affinity. However mutation of two conserved lysine residues adjacent to the finger motif reduced both RNA binding and annealing activities of NCp10. These findings suggest that the complexed NC zinc finger is not directly involved in RNA-protein interactions but more probably in a zinc dependent conformation of NC protein modulating viral protein-protein interactions, essential to the process of viral RNA selection and virion assembly. Then the NC zinc finger may cooperate to select the viral RNA genome to be packaged into virions.

L9 ANSWER 38 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1990:3604 BIOSIS

DOCUMENT NUMBER: BA89:3604

TITLE: HIV-1 REVERSE TRANSCRIPTASE SPECIFICALLY INTERACTS WITH THE ANTICODON DOMAIN OF ITS COGNATE PRIMER **TRANSFER RNA.**

AUTHOR(S): BARAT C; LULLIEN V; SCHATZ O; KEITH G; NUGEYRE M T; GRUENINGER-LEITCH F; BARRE-SINOUSSE F; LEGRICE S F J; DARLIX J L

CORPORATE SOURCE: LABO-RETRO CENT. DE RECHERCHE DE BIOCHIMIE ET GENETIQUE CELLULAIRES DU CNRS, 118 ROUTE DE NARBONNE, F-31062 TOULOUSE CEDEX, FRANCE.

SOURCE: EMBO (EUR MOL BIOL ORGAN) J, (1989) 8 (11), 3279-3286.

CODEN: EMJODG. ISSN: 0261-4189.

FILE SEGMENT: BA; OLD

LANGUAGE: English

AB The virion cores of the replication type 1 human immunodeficiency virus (HIV-1), a retrovirus, contain an RNA genome associated with nucleocapsid (NC) and reverse transcriptase (RT p66/p51) molecules. In *vitro* reconstructions of these complexes with purified components show that NC is required for efficient annealing of the primer tRNA<sup>Lys</sup>,3. In the absence of NC, HIV-1 RT is unable to retrotranscribe the viral RNA template from the **tRNA** primer. We demonstrate that the HIV-1 RT p66/p51 specifically binds to its cognate primer tRNA<sup>Lys</sup>,3 even in the presence of a 100-fold molar excess of other **tRNAs**. Cross-linking analysis of this interaction locates the contact site to a region within the heavily modified anti-codon domain of tRNA<sup>Lys</sup>,3.

L9 ANSWER 39 OF 44 MEDLINE

DUPLICATE 20

ACCESSION NUMBER: 87106837 MEDLINE

DOCUMENT NUMBER: 87106837 PubMed ID: 2433189

TITLE: Translation of homologous and heterologous messenger RNAs in a yeast cell-free system.

AUTHOR: Hussain I; Leibowitz M J

CONTRACT NUMBER: GM-32413 (NIGMS)

SOURCE: GENE, (1986) 46 (1) 13-23.

Journal code: 7706761. ISSN: 0378-1119.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

Searcher : Shears 308-4994

09/713687

ENTRY MONTH: 198703  
ENTRY DATE: Entered STN: 19900302  
Last Updated on STN: 19970203  
Entered Medline: 19870319

AB A stable mRNA-dependent cell-free translation system from *Saccharomyces cerevisiae*, prepared by a modification of the method of Hofbauer et al. [Eur. J. Biochem. 122 (1982) 199-203] was active in translation of exogenous homologous and heterologous mRNAs. Optimal translational activity required the addition of polyamines and yeast **tRNA**. The m transcript of the M segment of double-stranded RNA, synthesized in **vitro** using the killer virus-associated RNA polymerase, directed the synthesis of preprotoxin polypeptide (M-p32), which was immunologically identified using antitoxin antibody. Sindbis **virus capsid protein** and rabbit globin were also translated from their mRNAs. Translation was inhibited by puromycin, sparsomycin and anisomycin. Analogues of the 5'-terminal caps present on most eukaryotic mRNA molecules inhibited translation of added mRNAs, including capped mRNAs and the uncapped killer virus mRNA.

L9 ANSWER 40 OF 44 MEDLINE DUPLICATE 21  
ACCESSION NUMBER: 86068014 MEDLINE  
DOCUMENT NUMBER: 86068014 PubMed ID: 2999784  
TITLE: Direct mapping of adeno-associated **virus capsid proteins** B and C: a possible ACG initiation codon.  
AUTHOR: Becerra S P; Rose J A; Hardy M; Baroudy B M; Anderson C W  
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1985 Dec) 82 (23) 7919-23.  
Journal code: 7505876. ISSN: 0027-8424.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 198601  
ENTRY DATE: Entered STN: 19900321  
Last Updated on STN: 19970203  
Entered Medline: 19860106

AB The three major **capsid proteins** of adeno-associated **virus** type 2 (AAV2) virions are designated A, B, and C and have molecular sizes of 90, 72, and 60 kDa, respectively. These proteins are related, and genetic studies have shown they are encoded by a long open reading frame located in the right half of the genome. The coding capacity distal to the first ATG in this reading frame is only 503 amino acids (i.e., a protein about the size of protein C), but an open frame sequence devoid of ATG codons extends upstream for an additional 184 codons. Although the amino terminus of the C capsid protein is blocked, partial amino acid sequence analyses of peptides from C have confirmed that it is encoded within the portion of the reading frame distal to the first ATG at nucleotide (nt) location 2810. The amino terminus of the B capsid protein is not blocked, and its sequence begins with alanine. The triplet encoding this alanine lies 64 codons upstream from the initiation site for protein C and is immediately preceded by the threonine codon, ACG, at nt 2615. This

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ACG codon lies in the most favorable sequence context for protein synthesis initiation. All three AAV2 capsid proteins are labeled in **vitro** with formyl[35S]methionyl-tRNA<sup>f</sup>, indicating that synthesis of each protein is initiated independently. Our data suggest that the nt 2615 ACG codon directs the methionyl-tRNA-dependent initiation of the AAV2 B capsid protein. Proteins B and C may be synthesized from the same mRNA species and their relative abundance could be determined by the efficiencies of their respective initiation codons.

L9 ANSWER 41 OF 44 MEDLINE DUPLICATE 22  
ACCESSION NUMBER: 79007648 MEDLINE  
DOCUMENT NUMBER: 79007648 PubMed ID: 211268  
TITLE: Polyoma virus has three late mRNA's: one for each virion protein.  
AUTHOR: Siddell S G; Smith A E  
SOURCE: JOURNAL OF VIROLOGY, (1978 Aug) 27 (2) 427-31.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 197811  
ENTRY DATE: Entered STN: 19900314  
Last Updated on STN: 19970203  
Entered Medline: 19781129

AB Polyoma virus mRNA, isolated from the cytoplasm of 3T6 cells late after infection and purified by hybridization to HpaII fragment 3 of polyoma virus DNA, was separated on 50% formamide-containing sucrose density gradients, and the fractionated RNA was recovered and translated in **vitro**. Analysis of the cell-free products showed that the minor virion protein VP3 was synthesized from an mRNA sedimenting at approximately 18S between the 19S VP2 mRNA and the 16S VP1 mRNA. Other experiments showed that the VP2 and VP3 can be labeled with formyl methionine from initiator tRNA. We conclude that there are three late polyoma virus mRNA's, each directing the synthesis of only one **viral capsid protein**.

L9 ANSWER 42 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.  
ACCESSION NUMBER: 79030132 EMBASE  
DOCUMENT NUMBER: 1979030132  
TITLE: Characterization of the mRNA's for the polyoma **virus capsid proteins** VP1, VP2, and VP3.  
AUTHOR: Hunter T.; Gibson W.  
CORPORATE SOURCE: Tum. Virol. Lab., Salk Inst., San Diego, Calif. 92112, United States  
SOURCE: Journal of Virology, (1978) 28/1 (240-253).  
CODEN: JOVIAM  
COUNTRY: United States  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 047 Virology  
029 Clinical Biochemistry  
LANGUAGE: English

AB Polyadenylated cytoplasmic RNA from polyoma virus-infected cells can be translated in the wheat germ system to yield all three polyoma **virus capsid proteins**, VP1, VP2, and

VP3. The translation products of RNA selected from total cytoplasmic RNA of infected cells by hybridization to polyoma virus DNA showed a high degree of enrichment for VP1, VP2, and VP3. The identity of the in *vitro* products with authentic virion proteins was established in two ways. First, tryptic peptide maps of the in *vitro* products were found to be essentially identical to those of their in vivo counterparts. Second, the mobilities of the in *vitro* products on two-dimensional gels were the same as those of viral proteins labeled in vivo. VP1, VP2, and VP3 were all labeled with [35S]formylmethionine when they were synthesized in the presence of [35S]formylmethionyl-*tRNA*(f)(met). We determined the sizes of the polyadenylated mRNA's for VP1, VP2, and VP3 by fractionation on gels. The sizes of the major mRNA species for the capsid proteins are as follows: VP2, 8.5 x 10<sup>5</sup> daltons; VP3, 7.4 x 10<sup>5</sup> daltons; and VP1, 4.6 x 10<sup>5</sup> daltons. We conclude that all three **viral capsid proteins** are synthesized independently in *vitro*, that all three **viral capsid proteins** are virally coded, and that each of the capsid proteins has a discrete mRNA.

L9 ANSWER 43 OF 44 MEDLINE DUPLICATE 23  
 ACCESSION NUMBER: 77013481 MEDLINE  
 DOCUMENT NUMBER: 77013481 PubMed ID: 1067601  
 TITLE: Initiation of translation directed by 42S' and 26S RNAs from Semliki Forest virus in *vitro*.  
 AUTHOR: Glanville N; Ranki M; Morser J; Kaariainen L; Smith A E  
 SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1976 Sep) 73 (9) 3059-63.  
 Journal code: 7505876. ISSN: 0027-8424.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 197612  
 ENTRY DATE: Entered STN: 19900313  
 Last Updated on STN: 19900313  
 Entered Medline: 19761201

AB The proteins synthesized in *vitro* in response to 42S and 26S RNAs from Semliki Forest virus were labeled with formyl-[35S]methionine from initiator *tRNA*. One **protein** which comigrated with **viral capsid protein** was labeled under the direction of 26S RNA, and only one labeled peptide was detected after digestion with trypsin. Further digestion with pronase gave rise to the dipeptide fMet-AsN. Several labeled polypeptides were found in the 42S RNA directed product and these had molecular weights of up to 150,000. However, tryptic digestion of the product yielded only one formylmethionyl-labeled peptide, which had a different mobility from that directed by the 26S RNA. Further digestion with pronase gave a single dipeptide, fMet-Ala. This indicates that nonstructural proteins as large as 150,000 daltons are probably synthesized from one initiation site on the 42S RNA. Translation starting from the internal initiation site on the 42S RNA, which is equivalent to that on the 26S RNA, could not be detected under the conditions used. Internal initiation sites which are similarly inactive have also been detected in other viral RNAs (e.g., brome mosaic virus, tobacco

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mosaic virus, and polyoma 19S RNA) and this suggests that, although eukaryotic mRNAs can contain more than one initiation site for protein synthesis, only the site nearer the 5' terminus is active in vitro.

L9 ANSWER 44 OF 44 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.  
ACCESSION NUMBER: 76175635 EMBASE  
DOCUMENT NUMBER: 1976175635  
TITLE: Initiation sites for translation of Sindbis virus 42S and 26S messenger RNAs.  
AUTHOR: Cancedda R.; Villa Komaroff L.; Lodish H.F.; Schlesinger M.  
CORPORATE SOURCE: II Fac. Med., Univ. Naples, Italy  
SOURCE: Cell, (1975) 6/2 (215-222).  
CODEN: CELLB5  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 047 Virology  
022 Human Genetics  
LANGUAGE: English

AB Sindbis virus 26S RNA is the principal species of virus specific RNA found in the infected cell; it is derived from a one third segment of virion 42S RNA. When translated in cell free extracts from mouse ascites cells or rabbit reticulocytes, 26S RNA directed the synthesis primarily of the 33,000 dalton **virus capsid protein**, and the **protein** products were in the form of free peptides rather than peptidyl **tRNA**. In contrast, the polypeptides synthesized in either extract in response to Sindbis virus 42S RNA were heterogeneous, ranging in molecular weight from 33,000 to 190,000, and were largely in the form of peptidyl **tRNA**. The number of independent initiation sites on the 26S and 42S RNAs was determined by analyzing a tryptic digest of reaction products labeled with yeast N formyl 35S methionyl **tRNA**(F)(MET). The 26S RNA appeared to contain a single initiation site, and this site could also be found in varying amounts in different preparations of 42S RNA. However, a second initiation site, distinct from that of 26S RNA was the major site in 42S virion RNA. These results suggest that 42S virion RNA contains two potential sites for initiation of protein synthesis. Only one of these may be active, however, and it is postulated that the second site functions primarily, if not exclusively, in the subgenomic 26S RNA species. In this regard, Sindbis virus 42S RNA may represent a novel form of a eucaryotic messenger RNA.

=> fil hom  
FILE 'HOME' ENTERED AT 12:52:52 ON 08 OCT 2002



09/713687

(~~FILE=HCAPLUS~~ ENTERED AT 12:24:29 ON 08 OCT 2002)

L1 29954 SEA FILE=HCAPLUS ABB=ON PLU=ON TRNA OR (T OR TRANSFER?)  
(W) (RNA OR RIBONUCLEIC OR RIBO NUCLEIC)  
L5 4555 SEA FILE=HCAPLUS ABB=ON PLU=ON (PEPTIDE OR PROTEIN OR  
POLYPROTEIN OR POLYPEPTIDE) (5A) ((HCV OR HEPATIT? C OR  
FLAVIVIR? OR VIRUS OR VIRAL) (5A) (NUCLEOCAPSID? OR  
CAPSID? OR PSEUDONUCLEOCAPSID?))  
L6 54 SEA FILE=HCAPLUS ABB=ON PLU=ON L1 AND L5  
L7 25 SEA FILE=HCAPLUS ABB=ON PLU=ON L6 AND VITRO

L7 ANSWER 1 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:414964 HCAPLUS

DOCUMENT NUMBER: 137:180722

TITLE: Functional interactions of **nucleocapsid**  
**protein** of feline immunodeficiency  
**virus** and cellular prion **protein**  
with the viral RNA

AUTHOR(S): Moscardini, Mila; Pistello, Mauro; Bendinelli,  
M.; Ficheux, Damien; Miller, Jennifer T.; Gabus,  
Caroline; Le Grice, Stuart F. J.; Surewicz,  
Witold K.; Darlix, Jean-Luc

CORPORATE SOURCE: Department of Biomedicine, University of Pisa,  
Pisa, I-56127, Italy

SOURCE: Journal of Molecular Biology (2002), 318(1),  
149-159

CODEN: JMOBAK; ISSN: 0022-2836

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB All lentiviruses and oncoretroviruses examd. so far encode a major  
nucleic-acid binding protein (nucleocapsid or NC\* protein), approx.  
2500 mols. of which coat the dimeric RNA genome. Studies on HIV-1  
and MoMuLV using in **vitro** model systems and in vivo have  
shown that NC protein is required to chaperone viral RNA  
dimerization and packaging during virus assembly, and proviral DNA  
synthesis by reverse transcriptase (RT) during infection. The human  
cellular prion protein (PrP), thought to be the major component of  
the agent causing transmissible spongiform encephalopathies (TSE),  
was recently found to possess a strong affinity for nucleic acids  
and to exhibit chaperone properties very similar to HIV-1 NC protein  
in the HIV-1 context in **vitro**. Tight binding of PrP to  
nucleic acids is proposed to participate directly in the prion  
disease process. To extend our understanding of lentiviruses and of  
the unexpected nucleic acid chaperone properties of the human prion  
protein, we set up an in **vitro** system to investigate  
replication of the feline immunodeficiency virus (FIV), which is  
functionally and phylogenetically distant from HIV-1. The results  
show that in the FIV model system, NC protein chaperones viral RNA  
dimerization, primer tRNA<sup>Lys</sup>, 3 annealing to the genomic  
primer-binding site (PBS) and minus strand DNA synthesis by the  
homologous FIV RT. FIV NC protein is able to trigger specific viral  
DNA synthesis by inhibiting self-priming of reverse transcription.  
The human prion protein was found to mimic the properties of FIV NC  
with respect to primer tRNA annealing to the viral RNA and  
chaperoning minus strand DNA synthesis.

REFERENCE COUNT: 48 THERE ARE 48 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

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L7 ANSWER 2 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2002:357237 HCAPLUS

DOCUMENT NUMBER: 137:89946

TITLE: Conformational Changes Accompanying  
Self-Assembly of the Hepatitis C Virus Core  
Protein

AUTHOR(S): Kunkel, Meghan; Watowich, Stanley J.

CORPORATE SOURCE: Department of Human Biological Chemistry &  
Genetics and the Sealy Center for Structural  
Biology, University of Texas Medical Branch,  
Galveston, TX, 77555, USA

SOURCE: Virology (2002), 294(2), 239-245

CODEN: VIRLAX; ISSN: 0042-6822

PUBLISHER: Elsevier Science

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Although a no. of recent studies have suggested that the function of the hepatitis C virus (HCV) core protein may be both to package the viral genome and to modulate host cellular processes, little is known of the structure of the core protein necessary to accomplish these functions. Using in **vitro** assembled particles that mimic essential features of native HCV nucleocapsids, we report the earliest structural information of the **HCV core protein** and its **nucleocapsid**. The core protein is proteinase-resistant when assembled into nucleocapsid-like particles or complexed with nucleic acid in **vitro**. In contrast, the highly basic amino terminus of the free core protein is sensitive to proteolytic digestion. The hydrophobic carboxyl-terminal region of the core protein stabilizes the structure of the free core protein but is not required to stabilize core protein assembled into nucleocapsid-like particles or complexed to nucleic acid. Significantly, the carboxyl-terminal region is sufficient, but not necessary, to fold the core protein into a stable structure. These data are consistent with a model of a partially flexible HCV core protein that undergoes extensive conformational changes upon binding to nucleic acid and assembling into nucleocapsid particles. In addn., the susceptibility of nucleocapsid particles to RNase digestion suggests that RNA-core interactions may stabilize HCV nucleocapsids.

REFERENCE COUNT: 26 THERE ARE 26 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L7 ANSWER 3 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:352529 HCAPLUS

DOCUMENT NUMBER: 133:262220

TITLE: In **vitro** studies on **tRNA**  
annealing and reverse transcription with mutant  
HIV-1 RNA templates

AUTHOR(S): Beerens, Nancy; Berkhout, Ben

CORPORATE SOURCE: Department of Human Retrovirology, Academic  
Medical Center, University of Amsterdam,  
Amsterdam, 1100 DE, Neth.

SOURCE: Journal of Biological Chemistry (2000), 275(20),  
15474-15481

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular

Searcher : Shears 308-4994

09/713687

DOCUMENT TYPE: Biology  
Journal  
LANGUAGE: English

AB The human immunodeficiency virus type 1 (HIV-1) RNA genome encodes a semistable stem-loop structure, the U5-PBS hairpin, which occludes part of the **tRNA** primer binding site (PBS). In previous studies, we demonstrated that mutations that alter the stability of the U5-PBS hairpin inhibit virus replication. A reverse transcription defect was measured in assays with the virion-extd. RNA-**tRNA** complexes. We now extend these studies with in **vitro** synthesized wild-type and mutant RNA templates that were tested in primer annealing and reverse transcription assays. The effect of annealing temp. and the presence of the **viral nucleocapsid protein** on reverse transcription was analyzed for the templates with a stabilized or destabilized U5-PBS hairpin, and in reactions initiated by **tRNA** or DNA primers. The results of this in **vitro** assay are consistent with the in vivo findings, in that both **tRNA** annealing and initiation of reverse transcription are sensitive to stable template RNA structure. Reverse transcription initiated by a DNA primer is less hindered by secondary structure in the RNA template than **tRNA** primed reactions. The inhibitory effect of template structure on **tRNA**-primed reverse transcription is more pronounced in this in **vitro** assay compared with the in vivo material, indicating that the heat-annealed RNA-**tRNA** complex differs from the virion-extd. viral RNA-**tRNA** complex.

REFERENCE COUNT: 49 THERE ARE 49 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L7 ANSWER 4 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:275903 HCAPLUS

DOCUMENT NUMBER: 133:40377

TITLE: Nucleic acid-dependent cross-linking of the  
**nucleocapsid protein** of  
Sindbis virus

AUTHOR(S): Tellinghuisen, Timothy L.; Kuhn, Richard J.

CORPORATE SOURCE: Department of Biological Sciences, Purdue  
University, West Lafayette, IN, 47907, USA

SOURCE: Journal of Virology (2000), 74(9), 4302-4309  
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The assembly of the alphavirus nucleocapsid core is a multistep event requiring the assocn. of the nucleocapsid protein with nucleic acid and the subsequent oligomerization of capsid proteins into an assembled core particle. Although the mechanism of assembly has been investigated extensively both in vivo and in **vitro**, no intermediates in the core assembly pathway have been identified. Through the use of both truncated and mutant Sindbis virus **nucleocapsid proteins** and a variety of crosslinking reagents, a possible nucleic acid-protein assembly intermediate has been detected. The cross-linked species, a covalent dimer, has been detected only in the presence of nucleic acid and with capsid proteins capable of binding nucleic acid. Optimum nucleic acid-dependent crosslinking was seen at a

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protein-to-nucleic-acid ratio identical to that required for max. binding of the capsid protein to nucleic acid. Identical results were obsd. when crosslinking in **vitro** assembled core particles of both Sindbis and Ross River viruses. Purified cross-linked dimers of truncated proteins and of mutant proteins that failed to assemble were found to incorporate into assembled core particles when present as minor components in assembly reactions, suggesting that the crosslinking traps an authentic intermediate in nucleocapsid core assembly. Endoproteinase Lys-C mapping of the position of the cross-link indicated that lysine 250 of one capsid protein was cross-linked to lysine 250 of an adjacent capsid protein. Examn. of the position of the cross-link in relation to the existing model of the nucleocapsid core suggests that the cross-linked species is a cross-capsomere contact between a pentamer and hexamer at the quasi-threefold axis or is a cross-capsomere contact between hexamers at the threefold axis of the icosahedral core particle and suggests several possible assembly models involving a nucleic acid-bound dimer of capsid protein as an early step in the assembly pathway.

REFERENCE COUNT: 30 THERE ARE 30 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L7 ANSWER 5 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 2000:77926 HCAPLUS

DOCUMENT NUMBER: 132:217923

TITLE: In **vitro** studies of human **tRNA**  
(lys,3) primer annealing by human  
immunodeficiency **virus** type 1  
**nucleocapsid protein**

AUTHOR(S): Chan, Chankong; Barden

CORPORATE SOURCE: Univ. of Minnesota, Minneapolis, MN, USA

SOURCE: (1999) 152 pp. Avail.: UMI, Order No. DA9934942  
From: Diss. Abstr. Int., B 1999, 60(6), 2668

DOCUMENT TYPE: Dissertation

LANGUAGE: English

AB Unavailable

L7 ANSWER 6 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:598851 HCAPLUS

DOCUMENT NUMBER: 131:320081

TITLE: Characterization of the block in replication of  
nucleocapsid protein zinc finger mutants from  
Moloney murine leukemia virus

AUTHOR(S): Gorelick, Robert J.; Fu, William; Gagliardi,  
Tracy D.; Bosche, William J.; Rein, Alan;  
Henderson, Louis E.; Arthur, Larry O.

CORPORATE SOURCE: AIDS Vaccine Program, SAIC Frederick, National  
Cancer Institute Frederick Cancer Research and  
Development Center, Frederick, MD, 21702-1201,  
USA

SOURCE: Journal of Virology (1999), 73(10), 8185-8195

CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Mutagenesis studies have shown that retroviral nucleocapsid (NC)  
protein Zn<sup>2+</sup> fingers (-Cys-X2-Cys-X4-His-X4-Cys- [CCHC]) perform

Searcher : Shears 308-4994

multiple functions in the virus life cycle. Moloney murine leukemia virus mutants His 34.fwdarw.Cys (CCCC) and Cys 39.fwdarw.His (CCHH) were able to package their genomes normally but were replication defective. Thermal dissocn. expts. showed that the CCHH mutant was not defective in genomic RNA dimer structure. Primer **tRNA** placement on the viral genome and the ability of the **tRNA** to function in reverse transcription initiation in **vitro** also appear normal. Some "full-length" DNA copies of the viral genome were synthesized in mutant virus-infected cells. The CCCC and CCHH mutants produced these DNA copies at greatly reduced levels. Circle junction fragments, amplified from 2-long-terminal-repeat viral DNA (vDNA) by PCR, were cloned and characterized. VDNA isolated from cells infected with mutant virions had a wide variety of abnormalities at the site at which the 2 ends of the linear precursor had been ligated to form the circle (i.e., the junction between the 5' end of U3 and the 3' end of U5). In some mols., bases were missing from regions corresponding to the U3 and U5 linear vDNA termini; in others, the viral sequences extended either beyond the U5 sequences into the primer-binding site and 5' leader or beyond the U3 sequences into the polypurine tract into the env coding region. Still other mols. contained nonviral sequences between the linear vDNA termini. Such defective genomes would certainly be unsuitable substrates for integration. Thus, strict conservation of the CCHC structure in NC is required for infection events prior to and possibly including integration.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L7 ANSWER 7 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:263474 HCAPLUS

DOCUMENT NUMBER: 131:98386

TITLE: The human immunodeficiency virus type 1 Gag polyprotein has nucleic acid chaperone activity: possible role in dimerization of genomic RNA and placement of **tRNA** on the primer binding site

AUTHOR(S): Feng, Ya-Xiong; Campbell, Stephen; Harvin, Demetria; Ehresmann, Bernard; Ehresmann, Chantal; Rein, Alan

CORPORATE SOURCE: Retroviral Genetics Section, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, 21702, USA

SOURCE: Journal of Virology (1999), 73(5), 4251-4256  
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The formation of an infectious retrovirus particle requires several RNA-RNA interaction events. In particular, the genomic RNA mols. form a dimeric structure, and a cellular **tRNA** mol. is annealed to an 18-base complementary region (the primer binding site, or PBS) on the genomic RNA, where it will serve as primer for reverse transcription. **TRNAs** normally possess a highly stable secondary and tertiary structure; it seems unlikely that annealing of a **tRNA** mol. to the PBS, which involves unwinding of this structure, could occur efficiently at physiol.

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temps. without the assistance of a cofactor. Many prior studies have shown that the **viral nucleocapsid** (NC) **protein** can act as a nucleic acid chaperone (i.e., facilitate annealing events between nucleic acids), and the assays used to demonstrate this activity include its ability to catalyze dimerization of transcripts representing retroviral genomes and the annealing of **tRNA** to the PBS in **vitro**. However, mature NC is not required for these events in vivo, since protease-deficient viral mutants, in which NC is not cleaved from the parental Gag polyprotein, are known to contain dimeric RNAs with **tRNA** annealed to the PBS. In the present expts., we have tested recombinant human immunodeficiency virus type 1 Gag polyprotein for nucleic acid chaperone activity. The protein was pos. by all of our assays, including the ability to stimulate dimerization and to anneal **tRNA** to the PBS in **vitro**. In quant. expts., its activity was approx. equiv. on a molar basis to that of NC. Based on these results, we suggest that the Gag polyprotein (presumably by its NC domain) catalyzes the annealing of **tRNA** to the PBS during (or before) retrovirus assembly in vivo.

REFERENCE COUNT: 36 THERE ARE 36 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L7 ANSWER 8 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:172329 HCAPLUS

DOCUMENT NUMBER: 130:307463

TITLE: Role of post-transcriptional modifications of  
primer tRNA<sup>Lys</sup>,3 in the fidelity and efficacy of  
plus strand DNA transfer during HIV-1 reverse  
transcription

AUTHOR(S): Auxilien, Sylvie; Keith, Gerard; Le Grice,  
Stuart F. J.; Darlix, Jean-Luc

CORPORATE SOURCE: LaboRetro ENS, INSERM U412, Lyon, 69364, Fr.

SOURCE: Journal of Biological Chemistry (1999), 274(7),  
4412-4420

CODEN: JBCHA3; ISSN: 0021-9258

PUBLISHER: American Society for Biochemistry and Molecular  
Biology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB During HIV reverse transcription, (+) strand DNA synthesis is primed by an RNase H-resistant sequence, the polypurine tract, and continues as far as a 18-nt double-stranded RNA region corresponding to the 3' end of tRNA<sup>Lys</sup>,3 hybridized to the viral primer binding site (PBS). Before (+) strand DNA transfer, reverse transcriptase (RT) needs to unwind the double-stranded **tRNA**-PBS RNA in order to reverse-transcribe the 3' end of primer tRNA<sup>Lys</sup>,3. Since the detailed mechanism of (+) strand DNA transfer remains incompletely understood, we developed an in **vitro** system to closely examine this mechanism, composed of HIV 5' RNA, natural modified tRNA<sup>Lys</sup>,3, synthetic unmodified tRNA<sup>Lys</sup>,3 or oligonucleotides (RNA or DNA) complementary to the PBS, as well as the **viral proteins** RT and **nucleocapsid protein** (NCp7). Prior to (+) strand DNA transfer, RT stalls at the double-stranded **tRNA**-PBS RNA complex and is able to reverse-transcribe modified nucleosides of natural tRNA<sup>Lys</sup>,3. Modified nucleoside m1A-58 of natural tRNA<sup>Lys</sup>,3 is only partially

effective as a stop signal, as RT can transcribe as far as the hyper-modified adenosine (ms2t6A-37) in the anticodon loop. M1A-58 is almost always transcribed into A, whereas other modified nucleosides are transcribed correctly, except for m7G-46, which is sometimes transcribed into T. In contrast, synthetic tRNA<sup>Lys</sup>,3, an RNA PBS primer, and a DNA PBS primer are completely reverse-transcribed. In the presence of an acceptor template, (+) strand DNA transfer is efficient only with templates contg. natural tRNA<sup>Lys</sup>,3 or the RNA PBS primer. Sequence anal. of transfer products revealed frequent errors at the transfer site with synthetic tRNA<sup>Lys</sup>,3, not obsd. with natural tRNA<sup>Lys</sup>,3. Thus, modified nucleoside m1A-58, present in all retroviral **tRNA** primers, appears to be important for both efficacy and fidelity of (+) strand DNA transfer. We show that other factors such as the nature of the (-) PBS of the acceptor template and the RNase H activity of RT also influence the efficacy of (+) strand DNA transfer.

REFERENCE COUNT: 43 THERE ARE 43 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 9 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1999:167604 HCAPLUS

DOCUMENT NUMBER: 131:55342

TITLE: Binding Properties of the Human Immunodeficiency Virus Type 1 Nucleocapsid

**Protein** p7 to a Model RNA: Elucidation of the Structural Determinants for Function  
AUTHOR(S): Urbaneja, Maria A.; Kane, Bradley P.; Johnson, Donald G.; Gorelick, Robert J.; Henderson, Louis E.; Casas-Finet, Jose R.

CORPORATE SOURCE: AIDS Vaccine Program, SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, MD, 21702-1201, USA

SOURCE: Journal of Molecular Biology (1999), 287(1), 59-75

CODEN: JMOBAK; ISSN: 0022-2836

PUBLISHER: Academic Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB HIV-1 nucleocapsid protein (NCp7) is a double zinc-fingered protein that has been traditionally implicated in viral RNA recognition and packaging, in addn. to its tight assocn. with genomic RNA and **tRNA** primer within the virion nucleocapsid. The availability of large quantities of viral or recombinant wild-type NCp7 and mutant p7 has made possible the assignment of the different roles that structural motifs within the protein play during RNA binding. At low ionic strength binding to the homopolymeric fluorescent RNA, poly(.epsilon).A), is electrostatically driven and four sodium ions are displaced. Arg7 in the flanking N-terminal region, Lys20 and Lys26 in the first zinc finger and one pos. charged residue (attributed to Lys41) in the second zinc finger are involved in electrostatic contacts with RNA. The p7 zinc fingers do not function independently but concomitantly. The first zinc finger (both isolated or in the context of the full-length protein) has a more prominent electrostatic interaction than the second one. The second zinc finger dominates the non-electrostatic stabilization of

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the binding to RNA due to stacking of its Trp residue with nucleic acid bases. Mutations in the highly conserved retroviral Zn-coordinating residues (CCHC) to steroid hormone receptor (CCCC) or transcription factor (CCHH) metal cluster types do not affect RNA binding. In spite of the limited impact in RNA binding affinity in **vitro** or RNA packaging in vivo that such mutations or structural alterations impart, they impair or abolish virus infectivity. It is likely that such an effect stems from the involvement of NCp7 in crucial steps of the virus life cycle other than RNA binding. (c) 1999 Academic Press.

REFERENCE COUNT: 80 THERE ARE 80 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L7 ANSWER 10 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1998:578140 HCAPLUS

DOCUMENT NUMBER: 129:286701

TITLE: The yeast Ty3 retrotransposon contains a 5'-3'  
bipartite primer-binding site and encodes  
nucleocapsid protein NCp9 functionally  
homologous to HIV-1 NCp7

AUTHOR(S): Gabus, Caroline; Ficheux, Damien; Rau, Michael;  
Keith, Gerard; Sandmeyer, Suzanne; Darlix,  
Jean-Luc

CORPORATE SOURCE: LaboRetro, Unite de Virologie Humaine, INSERM  
(#412), Ecole Normale Supérieure de Lyon, Lyon,  
69364, Fr.

SOURCE: EMBO Journal (1998), 17(16), 4873-4880

CODEN: EMJODG; ISSN: 0261-4189

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Retroviruses, including HIV-1 and the distantly related yeast  
retroelement Ty3, all encode a nucleoprotein required for virion  
structure and replication. During an in **vitro** comparison  
of HIV-1 and Ty3 nucleoprotein function in RNA dimerization and cDNA  
synthesis, we discovered a bipartite primer-binding site (PBS) for  
Ty3 composed of sequences located at opposite ends of the genome.  
Ty3 cDNA synthesis requires the 3' PBS for primer tRNA<sup>i</sup>Met  
annealing to the genomic RNA, and the 5' PBS, in cis or in trans, as  
the reverse transcription start site. Ty3 RNA alone is unable to  
dimerize, but formation of dimeric tRNA<sup>i</sup>Met bound to the PBS was  
found to direct dimerization of Ty3 RNA-tRNA<sup>i</sup>Met. Interestingly,  
HIV-1 nucleocapsid protein NCp7 and Ty3 NCp9 were interchangeable  
using HIV-1 and Ty3 RNA template-primer systems. Our findings  
impact on the understanding of non-canonical reverse transcription  
as well as on the use of Ty3 systems to screen for anti-NCp7 drugs.

L7 ANSWER 11 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:802603 HCAPLUS

DOCUMENT NUMBER: 128:111499

TITLE: The nucleocapsid protein specifically anneals  
tRNA<sup>Lys</sup>-3 onto a noncomplementary primer binding  
site within the HIV-1 RNA genome in  
**vitro**

AUTHOR(S): Chan, Barden; Musier-Forsyth, Karin

CORPORATE SOURCE: Dep. Chem., Univ. Minnesota, Minneapolis, MN,  
55455, USA



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SOURCE: Proceedings of the National Academy of Sciences  
of the United States of America (1997), 94(25),  
13530-13535  
CODEN: PNASA6; ISSN: 0027-8424  
PUBLISHER: National Academy of Sciences  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB HIV type 1 (HIV-1) specifically uses host cell tRNA<sup>Lys</sup>-3 as a primer for reverse transcription. The 3' 18 nucleotides of this **tRNA** are complementary to a region on the HIV RNA genome known as the primer binding site (PBS). HIV-1 has a strong preference for maintaining a lysine-specific PBS in vivo, and viral genomes with mutated PBS sequences quickly revert to be complementary to tRNA<sup>Lys</sup>-3. To investigate the mechanism for the obsd. PBS reversion events in **vitro**, we examd. the capability of the nucleocapsid protein (NC) to anneal various **tRNA** primer sequences onto either complementary or noncomplementary PBSs. We show that NC can anneal different full-length **tRNAs** onto viral RNA transcripts derived from the HIV-1 MAL or HXB2 isolates, provided that the PBS is complementary to the **tRNA** used. In contrast, NC promotes specific annealing of only tRNA<sup>Lys</sup>-3 onto an RNA template (HXB2) whose PBS sequence has been mutated to be complementary to the 3' 18 nt of human tRNA<sup>Pro</sup>. Moreover, HIV-1 reverse transcriptase extends this binary complex from the proline-specific PBS. The formation of the noncomplementary binary complex does not occur when a chimeric tRNA<sup>Lys</sup>/Pro contg. proline-specific D and anticodon domains is used as the primer. Thus, elements outside the acceptor-T<sub>psi</sub>.C domains of tRNA<sup>Lys</sup>-3 play an important role in preferential primer use in **vitro**. Our results support the hypothesis that mutant PBS reversion is a result of tRNA<sup>Lys</sup>-3 annealing onto and extension from a PBS that specifies an alternate host cell **tRNA**.

L7 ANSWER 12 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1997:789372 HCAPLUS  
DOCUMENT NUMBER: 128:85609  
TITLE: Identification of a specific interaction between  
the coronavirus mouse hepatitis **virus**  
A59 **nucleocapsid protein** and  
packaging signal

AUTHOR(S): Molenkamp, Richard; Spaan, Willy J. M.  
CORPORATE SOURCE: Department of Virology, Institute of Medical  
Microbiology, Leiden University, AZL-L4-Q,  
Leiden, 2300 RC, Neth.

SOURCE: Virology (1997), 239(1), 78-86  
CODEN: VIRLAX; ISSN: 0042-6822  
PUBLISHER: Academic Press  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The coronavirus mouse hepatitis virus (MHV) is an enveloped pos. stranded RNA virus. In infected cells MHV produces a 3' coterminal nested set of subgenomic mRNAs. Only the genomic RNA, however, is encapsidated by the nucleocapsid protein and incorporated in infectious MHV virions. It is believed that an RNA packaging signal (Ps), present only in the genomic RNA, is responsible for this selectivity. Earlier studies mapped this signal to a 69-nt stem-loop structure positioned in the 3' end of ORF1b. The selective encapsidation mechanism probably initiates by specific

interaction of the packaging signal with the nucleocapsid protein. In this study we demonstrate the *in vitro* interaction of the MHV-A59 nucleocapsid protein with the packaging signal of MHV using gel retardation and UV crosslinking assays. This interaction was obsd. not only with the nucleocapsid protein from infected cells but also with that from purified virions and from cells expressing a recombinant nucleocapsid protein. The specificity of the interaction was demonstrated by competition expts. with nonlabeled Ps contg. RNAs, **tRNA**, and total cytoplasmic RNA. The results indicated that no virus specific modification of the N protein or the presence of other viral proteins are required for this *in vitro* interaction. The assays described in this report provide us with a powerful tool for studying encapsidation (initiation) in more detail.

L7 ANSWER 13 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:433036 HCAPLUS

DOCUMENT NUMBER: 125:134569

TITLE: Human immunodeficiency **virus** type 2  
**nucleocapsid protein** (NCp7)  
directs specific initiation of minus-strand DNA  
synthesis primed by human tRNA<sup>3</sup>Lys in  
**vitro**: studies of viral RNA molecules  
mutated in regions that flank the primer binding  
site

AUTHOR(S): Li, Xuguang; Quan, Yudong; Arts, Eric J.; Li,  
Zhuo; Preston, Bradley D.; De Rocquigny, Hugues;  
Roques, Bernard P.; Darlix, Jean-Luc; Kleiman,  
Lawrence; et al.

CORPORATE SOURCE: Dep. Med and Microbiology, McGill University  
AIDS Center, Montreal, QC, Can.

SOURCE: Journal of Virology (1996), 70(8), 4996-5004  
CODEN: JOVIAM; ISSN: 0022-538X

PUBLISHER: American Society for Microbiology

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Retroviral reverse transcription starts near the 5' end of unspliced viral RNA at a sequence called the primer binding site (PBS), where the **tRNA** primer anneals to the RNA template for initiation of DNA synthesis. We have investigated the roles of NCp7 in annealing of primer tRNA<sup>3</sup>Lys to the PBS and in reverse transcriptase (RT) activity, using a cell-free reverse transcription reaction mixt. consisting of various 5' viral RNA templates, natural primer tRNA<sup>3</sup>Lys or synthetic primer, human immunodeficiency **virus** type 1 (HIV-1) **nucleocapsid protein** (NCp7), and HIV-1 RT. In the presence of tRNA<sup>3</sup>Lys, NCp7 was found to stimulate synthesis of minus-strand strong-stop DNA [(-)ssDNA], consistent with previous reports. However, specific DNA synthesis was obsd. only at a NCp7/RNA ratio similar to that predicted to be present in virions. Moreover, at these concns., NCp7 inhibited the synthesis of nonspecific reverse-transcribed DNA products, which are initiated because of self-priming by RNA templates. In contrasts to results obtained with tRNA<sup>3</sup>Lys as primer, NCp7 inhibited the synthesis of (-)ssDNA products primed by an 18-nucleotide (nt) ribonucleotide (rPR), complementary to the PBS, even through rPR can initiate synthesis of such material in the absence of preannealing with NCp7. Primer placement band shift assays showed that NCp7 was necessary for efficient formation of the **tRNA**-RNA complex. In

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contrast, NCp7 was found to prevent formation of the rPR-RNA complex. Since NCp7 appears to exert opposite effects (annealing vs. dissocn.) on tRNA<sup>3</sup>Lys and rPR substrates, the non-PBS binding regions of the tRNA<sup>3</sup>Lys mol. may play a role in the annealing of **tRNA** to the template. We also investigated the roles of an A-rich loop upstream of the PBS, a 7-nt region immediately downstream of the PBS, and a 54-nt region that may prevent formation of the U5-leader stem prevented tRNA<sup>3</sup>Lys placement and priming, while deletions in the A-rich loop or the 7-nt sequence had relatively minor effects in this regard.

L7 ANSWER 14 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1996:358081 HCAPLUS  
DOCUMENT NUMBER: 125:28608  
TITLE: RNA binding properties of core protein of the flavivirus Kunjin  
AUTHOR(S): Khromykh, A. A.; Westaway, E. G.  
CORPORATE SOURCE: Sir Albert Sakzewski Virus Res. Cent., R. Children's Hosp., Brisbane, Australia  
SOURCE: Archives of Virology (1996), 141(3-4), 685-699  
CODEN: ARVIDF; ISSN: 0304-8608  
PUBLISHER: Springer  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Kunjin virus (KUN) C is a typical flavivirus core protein which is truncated in vivo to a mature form of 105 residues enriched in lysine and arginine. In order to study the possible assocn. of KUN C with RNA in **vitro**, several recombinant C proteins with specific deletions were prep'd., each fused at the N-terminus to glutathione-S-transferase (GST) and expressed in Escherichia coli. They were reacted with KUN RNA probes transcribed in **vitro** from cDNA representing the 5' untranslated region (5' UTR, 93 of 96 nucleotides), the 3' UTR (624 nucleotides), and the 5' UTR plus most of the C coding region (5' core, 440 nucleotides). Fusion protein C107 (incorporating mature C) bound strongly to all KUN RNA probes with apparent specificity, being completely resistant to inhibition by 800 mM NaCl, and to competition by a large excess of **tRNA**. In reactions with labeled KUN RNA probes putative binding sites were identified in the isolated N-terminal (32 residues) and C-terminal (26 residues) basic amino acid domains; this binding was strongly competed by unlabeled KUN UTR probes but weakly or not at all by **tRNA**. These small domains probably acted cooperatively in binding of mature C to KUN RNA probes. The KUN RNA-core protein binding reactions are similar to those reported with other **viral** coat or **capsid proteins** and **viral** RNAs.

L7 ANSWER 15 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1995:331981 HCAPLUS  
DOCUMENT NUMBER: 122:99989  
TITLE: Binding of the HIV-1 nucleocapsid protein to the primer tRNA<sup>3</sup>Lys, in **vitro**, is essentially not specific  
AUTHOR(S): Mely, Yves; de Rocquigny, Hugues; Sorinas-Jimeno, Monica; Keith, Gerard; Roques, Bernard P.; Marquet, Roland; Gerard, Dominique  
CORPORATE SOURCE: Fac. Pharmacie, Univ. Louis Pasteur Strasbourg I, Illkirch, F-67401, Fr.

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SOURCE: Journal of Biological Chemistry (1995), 270(4),  
1650-6  
CODEN: JBCHA3; ISSN: 0021-9258  
PUBLISHER: American Society for Biochemistry and Molecular  
Biology  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The **nucleocapsid protein** NCp7 of human immunodeficiency **virus**, type 1, is a key component in the viral life cycle. Since, the first common step of all reported NCp7 activities corresponds to a nucleic acid-binding step, the NCp7 binding parameters to the natural primer tRNA<sup>3</sup>Lys were investigated. Using NCp7 intrinsic fluorescence, the authors found that (i) in 0.1 M NaCl, NCp7 bound noncooperatively to tRNA<sup>3</sup>Lys with a  $K_{obs} = 3.2 \times 10^6$  M<sup>-1</sup> assocn. constant and a binding site size, (ii) four ionic interactions were formed in the NCp7.tRNA<sup>3</sup>Lys complex, and (iii) nonelectrostatic factors provided about 60% of the binding energy. These binding parameters were not significantly altered when the natural tRNA<sup>3</sup>Lys was replaced by either an in **vitro** synthetic tRNA<sup>3</sup>Lys transcript, the heterologous yeast tRNA<sup>Phe</sup> or the structurally unrelated 5 S RNA from Escherichia coli. Moreover, the environment of the intrinsic fluorescent reporters (Trp37 and Trp61) was similar in the various complexes. Finally, expts. performed at low protein concn. provide no evidence of high affinity binding sites. Taken together, the data strongly suggested an essentially nonspecific binding of NCp7 to tRNA<sup>3</sup>Lys and thus did not seem to support a direct role of NCp7, per se, in the selection of tRNA<sup>3</sup>Lys from the pool of cellular **tRNAs**.

L7 ANSWER 16 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:698950 HCAPLUS  
DOCUMENT NUMBER: 121:298950  
TITLE: Monoclonal antibody-mediated inhibition of RNA binding and annealing activities of HIV type 1 nucleocapsid protein  
AUTHOR(S): Tanchou, Valerie; Delaunay, Thierry; Rocquigny, Hugues De; Bodeus, Monique; Darlix, Jean-Luc; Roques, Bernard; Benarous, Richard  
CORPORATE SOURCE: Institut Cochin de Genetique Moleculaire, Universite Rene Descartes, Paris, 75014, Fr.  
SOURCE: AIDS Research and Human Retroviruses (1994), 10(8), 983-93  
CODEN: ARHRE7; ISSN: 0889-2229  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Retroviral nucleocapsid (NC) proteins are highly basic, with one or two zinc fingers, and are required for virion formation, genomic RNA dimerization and packaging, and replication primer **tRNA** annealing to the viral RNA. The authors report here the first characterization of monoclonal antibodies directed against a retroviral nucleocapsid protein and their use to study the structure-function relationships of the nucleocapsid protein NCp7 of HIV-1. Four anti-NCp7 monoclonal antibodies (MAbs) have been generated by using NCp7 of HIV-1. The epitope targets of these MAbs were mapped using ELISA and BIAcore techniques. Whereas three of them are specific for epitopes located in the N and C termini of NCp7, the fourth one appears to be conformation specific. Only two of these MAbs, the conformation-specific one and the MAb recognizing

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an N-terminal epitope are able to inhibit the RNA-binding and annealing activities of NCp7 as well as strong-stop DNA synthesis in **vitro**. The binding of the two other MAb's onto NCp7 either has no effect or enhances the NCp7-RNA interactions. These MAb's also display a differential recognition of the Gag polyprotein precursor, which makes them useful tools for studying NC protein maturation in the course of virion morphogenesis.

L7 ANSWER 17 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:673837 HCAPLUS

DOCUMENT NUMBER: 121:273837

TITLE: Amino acid requirements of the nucleocapsid protein of HIV-1 for increasing catalytic activity of a Ki-ras ribozyme in **vitro**

AUTHOR(S): Mueller, Gerd; Strack, Bettina; Dannull, Jens; Sproat, Brian S.; Surovoy, Andrej; Jung, Gunther; Moelling, Karin

CORPORATE SOURCE: Max-Planck-Inst. Molekulare Genetik, Berlin, D-14195, Germany

SOURCE: Journal of Molecular Biology (1994), 242(4), 422-9

CODEN: JMOBAK; ISSN: 0022-2836

PUBLISHER: Academic

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The nucleocapsid protein NCp7 of HIV-1 is a single-stranded nucleic acid binding protein with several functions such as specific recognition, dimerization and packaging of viral RNA, **tRNA** annealing to viral RNA and protection against nucleases. Since some of these functions involve annealing and double-stranded RNA-melting activity we applied the nucleocapsid protein to a hammerhead ribozyme specific for the activated Ki-ras mRNA in **vitro**, which carries at its mutated codon 12 a GUU site. A synthetic ribozyme contg. 2'-O-allyl-modified nucleotides and alternatively in **vitro** transcribed ribozymes were used. At a one to one molar ratio of substrate to ribozyme almost no cleavage is obsd. at 37.degree.. Presence of a synthetic nucleocapsid protein significantly increases the catalytic activity of the ribozyme. Kinetic analyses by means of single and multiple turnover reactions performed at various substrate to ribozyme ratios lead to only a slight stimulation of the rate consts. for single turnover reactions. The rate consts. in multiple turnover reactions, however, are stimulated up to 17-fold by the presence of the nucleocapsid protein. The activating region of the nucleocapsid protein was characterized by a no. of mutants. The mutants demonstrate that activation requires both basic amino acid clusters as evidenced by point mutations. Deletion mutants indicate that the second zinc finger is totally dispensable and that replacement of the first zinc finger by a glycine-glycine spacer only slightly reduces the enhancing effect of the nucleocapsid protein on the ribozyme.

L7 ANSWER 18 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:466114 HCAPLUS

DOCUMENT NUMBER: 119:66114

TITLE: Identification of a binding site for the human immunodeficiency virus type 1 nucleocapsid protein

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AUTHOR(S): Sakaguchi, Kazuyasu; Zambrano, Nicola; Baldwin, Eric T.; Shapiro, Bruce A.; Erickson, John W.; Omichinski, James G.; Clore, G. Marius; Gronenborn, Angela M.; Appella, Ettore  
CORPORATE SOURCE: Lab. Cell Biol., Natl. Cancer Inst., Bethesda, MD, 20892, USA  
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (1993), 90(11), 5219-23  
CODEN: PNASA6; ISSN: 0027-8424  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB The nucleocapsid (NC) protein NCp7 of human immunodeficiency virus type 1 (HIV-1) is important for encapsidation of the virus genome, RNA dimerization, and primer **tRNA** annealing in **vitro**. Here evidence from gel mobility-shift expts. is presented indicating that NCp7 binds specifically to an RNA sequence. Two complexes were identified in native gels. The more slowly migrating complex contained two RNA mols. and one peptide, while the more rapidly migrating one is composed of one RNA and one peptide. Further, mutational anal. of the RNA shows that the predicted stem and loop structure of stem-loop 1 plays a crit. role. These results show that NCp7 binds to a unique RNA structure within the .psi. region; in addn., this structure is necessary for RNA dimerization. It is proposed that NCp7 binds to the RNA via a direct interaction of one zinc-binding motif to stem-loop 1 followed by binding of the other zinc-binding motif to stem-loop 1, stem-loop 2, or the linker region of the second RNA mol., forming a bridge between the two RNAs.

L7 ANSWER 19 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:229970 HCAPLUS  
DOCUMENT NUMBER: 118:229970  
TITLE: RNA binding of recombinant nucleocapsid proteins of hantaviruses  
AUTHOR(S): Goett, Peter; Stohwasser, Ralf; Schnitzler, Paul; Darai, Gholamreza; Bautz, Ekkehard K. F.  
CORPORATE SOURCE: ZMBH, Univ. Heidelberg, Heidelberg, 6900, Germany  
SOURCE: Virology (1993), 194(1), 332-7  
CODEN: VIRLAX; ISSN: 0042-6822  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Genes encoding the nucleocapsid (N) proteins of two hantaviruses, Hantaan virus strain 76-118 (HTN) and Puumala virus strain CG 18-20 (PUU), were expressed in Escherichia coli as histidine-tagged proteins. They were purified by metal-chelate affinity chromatog. under native or denaturing conditions to near homogeneity. The sol. form of HTN N protein was assocd. with RNA of E. coli. Renatured N proteins were shown to bind in **vitro** transcribed RNA representing the hantaviral small genomic (S) RNA segment. RNA binding was shown by affinity to filter-immobilized N proteins and by gel mobility shift assays. Competition expts. using **tRNA**, poly(U) and poly(A)+ U indicated that binding of RNA by the N protein is nonspecific. However, direct binding of dsRNA resulted in efficient formation of large complexes suggesting that double-stranded nucleic acids are bound preferentially. Carboxyterminal fragments of HTN and PUU N proteins contg. about 100

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amino acids of the carboxy termini retained full binding capacity indicating that RNA binding occurs via a carboxyterminal domain.

L7 ANSWER 20 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1991:530209 HCAPLUS

DOCUMENT NUMBER: 115:130209

TITLE: **Viral** RNA annealing activities of the **nucleocapsid protein** of Moloney murine leukemia **virus** are zinc independent

AUTHOR(S): Prats, Anne Catherine; Housset, Valerie; De Billy, Gerard; Cornille, Fabrice; Prats, Herve; Roques, Bernard; Darlix, Jean Luc

CORPORATE SOURCE: Cent. Rech. Biochim. Genet. Cell., CNRS, Toulouse, 31062, Fr.

SOURCE: Nucléic Acids Research (1991), 19(13), 3533-41  
CODEN: NARHAD; ISSN: 0305-1048

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The zinc fingers of retroviral gag nucleocapsid proteins (NC) are required for the specific packaging of the dimeric RNA genome into virions. In **vitro**, NC proteins activate both dimerization of viral RNA and annealing of the replication primer **tRNA** onto viral RNA, two reactions necessary for the prodn. of infectious virions. In this study the role of the zinc finger of Moloney murine leukemia virus (MoMuLV) NCp10 in RNA binding and annealing activities was investigated through modification or replacement of residues involved in zinc coordination. These alterations did not affect the ability of NCp10 to bind RNA and promote RNA annealing in **vitro**, despite a complete loss of zinc affinity. However mutation of two conserved lysine residues adjacent to the finger motif reduced both RNA binding and annealing activities of NCp10. These findings suggest that the complexed NC zinc finger is not directly involved in RNA-protein interactions but more probably in a zinc dependent conformation of NC protein modulating viral protein-protein interactions, essential to the process of viral RNA selection and virion assembly. Then the NC zinc finger may cooperate to select the viral RNA genome to be packaged into virions.

L7 ANSWER 21 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1989:73871 HCAPLUS

DOCUMENT NUMBER: 110:73871

TITLE: Cell-free system for protein formation from template mRNA containing ribosomes, amino acids, ATP, and GTP

INVENTOR(S): Alakhov, Yu. B.; Baranov, V. I.; Ovodov, S. Yu.; Ryabova, L. A.; Spirin, A. S.

PATENT ASSIGNEE(S): Institute of Protein, Academy of Sciences, U.S.S.R., USSR

SOURCE: PCT Int. Appl., 19 pp.  
CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: Russian

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.

KIND DATE

APPLICATION NO. DATE

Searcher : Shears 308-4994

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WO 8808453	A1	19881103	WO 1988-SU78	19880414
W: BG, FI, HU, JP, US				
RW: AT, BE, CH, DE, FR, GB, IT, LU, NL, SE				
SU 1441787	A1	19900923	SU 1987-4239148	19870429
EP 312617	A1	19890426	EP 1988-904712	19880414
EP 312617	B1	19930303		
R: AT, BE, CH, DE, FR, GB, IT, LI, LU, NL, SE				
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JP 07110236	B4	19951129		
HU 54420	A2	19910228	HU 1988-3398	19880414
AT 86306	E	19930315	AT 1988-904712	19880414
FI 8806002	A	19881228	FI 1988-6002	19881228
PRIORITY APPLN. INFO.:			SU 1987-4239148	19870429
			EP 1988-904712	19880414
			WO 1988-SU78	19880414

AB A cell-free system contg. ATP, GTP, and amino acids is described for the in **vitro** translation of mRNA to obtain, in high yield, proteins contg. 15-70 amino acids. The system contains ATP, GTP, 20 free amino acids, the mRNA to be translated, and ribosomes (e.g. from Escherichia coli or wheat embryo ext.). The mRNA for the capsid protein of phage MS2 was translated in a cell-free system contg. E. coli ribosomes in the presence of ATP, GTP, MS2 **tRNA**, and 20 amino acids. After 20 min incubation at 37.degree. the E. coli ribosomes synthesized 6000 pmol capsid protein (100 pmol cellular product/pmol capsid mRNA). Addn. of salmon calcitonin mRNA to the translation system resulted in formation of 18,000 pmol calcitonin (300 pmol cellular prooduct/pmol calcitonin mRNA). Optionally, wheat germ ext. was used in place of E. coli ribosomes to translate brome mosaic **virus** (BMV) **capsid protein** mRNA. After 20 h incubation 10,000 pmol BMV capsid protein was recovered (100 pmol cell product/pmol BMV mRNA). When salmon calcitonin mRNA was added to this system 15,000 pmol calcitonin was recovered (150 pmol cell product/pmol calcitonin mRNA).

L7 ANSWER 22 OF 25 HCAPLUS COPYRIGHT 2002 ACS  
 ACCESSION NUMBER: 1987:152484 HCAPLUS  
 DOCUMENT NUMBER: 106:152484  
 TITLE: Translation of homologous and heterologous messenger RNAs in a yeast cell-free system  
 AUTHOR(S): Hussain, Iffat; Leibowitz, Michael J.  
 CORPORATE SOURCE: Robert Wood Johnson Med. Sch., UMDNJ, Piscataway, NJ, 08854, USA  
 SOURCE: Gene (1986), 46(1), 13-23  
 CODEN: GENED6; ISSN: 0378-1119  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English

AB A stable mRNA-dependent cell-free translation system from Saccharomyces cerevisiae, prepd. by a modification of the method of R. Hofbauer et al. (1982) was active in translation of exogenous homologous and heterologous mRNAs. Optimal translational activity required the addn. of polyamines and yeast **tRNA**. The m transcript of the M segment of double-stranded RNA, synthesized in **vitro** using the killer virus-assocd. RNA polymerase, directed the synthesis of preprotoxin polypeptide (M-p32), which was immunol. identified using antitoxin antibody. Sindbis **virus capsid protein** and rabbit globin were also



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translated from their mRNAs. Translation was inhibited by puromycin, sparsomycin, and anisomycin. Analogs of the 5'-terminal caps present on most eukaryotic mRNA mols. inhibited translation of added mRNAs, including capped mRNAs and the uncapped killer virus mRNA.

L7 ANSWER 23 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1986:46420 HCAPLUS

DOCUMENT NUMBER: 104:46420

TITLE: Direct mapping of adeno-associated **virus capsid proteins** B and C: a possible ACG initiation codon

AUTHOR(S): Becerra, S. Patricia; Rose, James A.; Hardy, Medora; Baroudy, Bahige M.; Anderson, Carl W.

CORPORATE SOURCE: Lab. Biol. Viruses, Natl. Inst. Allergy Infect. Dis., Bethesda, MD, 20205, USA

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1985), 82(23), 7919-23

CODEN: PNASA6; ISSN: 0027-8424

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The 3 major **capsid proteins** of adeno-assocd.

**virus** type 2 (AAV2) virions are designated A, B, and C and have mol. sizes of 90, 72, and 60 kDa, resp. These proteins are related, and genetic studies have shown that they are encoded by a long open reading frame located in the right half of the genome. The coding capacity distal to the 1st ATG in this reading frame is only 503 amino acids (i.e., a protein about the size of protein C), but an open frame sequence devoid of ATG codons extends upstream for an addnl. 184 codons. Although the N terminus of the C capsid protein is blocked, partial amino acid sequence analyses of peptides from C have confirmed that it is encoded within the portion of the reading frame distal to the 1st ATG at nucleotide (nt) location 2810. The N terminus of the B capsid protein is not blocked, and its sequence begins with alanine. The triplet encoding this alanine lies 64 codons upstream from the initiation site for protein C and is immediately preceded by the threonine codon, ACG, at nucleotide 2615. This ACG codon lies in the most favorable sequence context for protein synthesis initiation. All 3 AAV2 capsid proteins are labeled in **vitro** with formyl[35S]methionyl-tRNA<sup>f</sup>, indicating that synthesis of each protein is initiated independently. Apparently, the nucleotide 2615 ACG codon directs the methionyl-tRNA-dependent initiation of the AAV2B capsid protein. Proteins B and C may be synthesized from the same mRNA species, and their relative abundance could be detd. by the efficiencies of their resp. initiation codons.

L7 ANSWER 24 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1978:543231 HCAPLUS

DOCUMENT NUMBER: 89:143231

TITLE: Polyoma virus has three late mRNA's: one for each virion protein

AUTHOR(S): Siddell, Stuart G.; Smith, Alan E.

CORPORATE SOURCE: Transl. Lab., Imperial Cancer Res. Fund, London, Engl.

SOURCE: J. Virol. (1978), 27(2), 427-31

CODEN: JOVIAM; ISSN: 0022-538X

DOCUMENT TYPE: Journal

Searcher : Shears 308-4994

09/713687

LANGUAGE: English

AB Polyoma virus mRNA, from the cytoplasm of 3T6 cells late after infection and purified by hybridization to HpaII fragment 3 of polyoma virus DNA, was sepd. on 50% formamide-contg. sucrose d. gradients, and the fractionated RNA was recovered and translated in **vitro**. Anal. of the cell-free products showed the minor virion protein VP3 was synthesized from an mRNA sedimenting at .apprx.18S between the 19S VP2 mRNA and the 16S VP1 mRNA. The VP2 and VP3 could be labeled with formylmethionine from initiator **tRNA**. Apparently there are 3 late polyoma virus mRNAs, each directing the synthesis of only 1 **viral capsid protein**.

L7 ANSWER 25 OF 25 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1976:556399 HCAPLUS

DOCUMENT NUMBER: 85:156399

TITLE: Initiation of translation directed by 42S and 26S RNAs from Semliki Forest virus in **vitro**

AUTHOR(S): Glanville, Niall; Ranki, Marjut; Morser, John; Kaariainen, Leevi; Smith, Alan E.

CORPORATE SOURCE: Dep. Virol., Univ. Helsinki, Helsinki, Finland

SOURCE: Proc. Natl. Acad. Sci. U. S. A. (1976), 73(9), 3059-63

CODEN: PNASA6

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The proteins synthesized in **vitro** in response to 42S and 26S RNAs from Semliki Forest virus were labeled with formylmethionine-35S from initiator **tRNA**. One **protein** which comigrated with **viral capsid protein** was labeled under the direction of 26S RNA, and only 1 labeled peptide was detected after digestion with trypsin. Further digestion with Pronase gave rise to the dipeptide formylmethionine-asparagine. Several labeled polypeptides were found in the 42S RNA directed product and these had mol. wts. of up to 150,000. However, tryptic digestion of the product yielded only 1 formylmethionyl-labeled peptide, which had a different mobility from that directed by the 26S RNA. Further digestion with Pronase gave a single dipeptide, formylmethionine-alanine. This indicates that nonstructural proteins as large as 150,000 daltons are probably synthesized from 1 initiation site on the 42S RNA. Translation starting from the internal initiation site on the 42S RNA, which is equiv. to that on the 26S RNA, could not be detected under the conditions used. Internal initiation sites which are similarly inactive have also been detected in other viral RNAs (e.g., brome mosaic virus, tobacco mosaic virus, and polyoma 19S RNA) and this suggests that, although eukaryotic mRNAs can contain more than 1 initiation site for protein synthesis, only the site nearer the 5' terminus is active in **vitro**.

FILE MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JEST-EPLUS, JAPIO' ENTERED AT 12:39:02 ON 08 OCT 2002)

100 S L7

44 DUB-REM L8 (56 DUPLICATES REMOVED)

L9 ANSWER 1 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 2002:278978 SCISEARCH

09/713687

THE GENUINE ARTICLE: 533GG

TITLE: The Tat protein of human immunodeficiency virus type 1 (HIV-1) can promote placement of **tRNA** primer onto viral RNA and suppress later DNA polymerization in HIV-1 reverse transcription

AUTHOR: Kameoka M; Morgan M; Binette M; Russell R S; Rong L W; Guo X F; Mouland A; Kleiman L; Liang C; Wainberg M A (Reprint)

CORPORATE SOURCE: McGill Univ, Jewish Gen Hosp, AIDS Ctr, Lady Davis Inst, 3755 Cote Ste Catherine Rd, Montreal, PQ H3T 1E2, Canada (Reprint); McGill Univ, Jewish Gen Hosp, AIDS Ctr, Lady Davis Inst, Montreal, PQ H3T 1E2, Canada; McGill Univ, Dept Microbiol, Montreal, PQ H3A 2B4, Canada

COUNTRY OF AUTHOR: Canada

SOURCE: JOURNAL OF VIROLOGY, (APR 2002) Vol. 76, No. 8, pp. 3637-3645.  
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904 USA.  
ISSN: 0022-538X.

DOCUMENT TYPE: Article; Journal

LANGUAGE: English

REFERENCE COUNT: 51

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Human immunodeficiency virus type-1 Tat has been proposed to play a role in the regulation of reverse transcription. We previously demonstrated that wild-type Tat can augment viral infectivity by suppressing the reverse transcriptase (RT) reaction at late stages of the viral life cycle in order to prevent the premature synthesis of potentially deleterious viral DNA products. Here we have performed a detailed analysis of the cell-free reverse transcription reaction to elucidate the mechanism(s) whereby Tat can affect this process. Our results show that Tat can suppress nonspecific DNA elongation while moderately affecting the specific initiation stage of reverse transcription. In addition, Tat has an RNA-annealing activity and can promote the placement of **tRNA** onto viral RNA. This points to a functional homology between Tat and the **viral nucleocapsid (NC) protein** that is known to be directly involved in this process. Experiments using a series of mutant Tat proteins revealed that the cysteine-rich and core domains of Tat are responsible for suppression of DNA elongation, while each of the cysteine-rich, core, and basic domains, as well as a glutamine-rich region in the C-terminal domain, are important for the placement of **tRNA** onto the viral RNA genome. These results suggest that Tat can play at least two different roles in the RT reaction, i.e., suppression of DNA polymerization and placement of **tRNA** onto viral RNA. We believe that the first of these activities of Tat may contribute to the overall efficiency of reverse transcription of the viral genome during a new round of infection as well as to enhanced production of infectious viral particles. We hypothesize that the second activity, illustrating functional homology between Tat and NC, suggests a potential role for NC in the displacement of Tat during viral maturation.

L9 ANSWER 2 OF 44 MEDLINE  
ACCESSION NUMBER: 2002311005 MEDLINE  
DOCUMENT NUMBER: 22050818 PubMed ID: 12054775

DUPLICATE 1

Searcher : Shears 308-4994

09/713687

TITLE: Functional interactions of **nucleocapsid protein** of feline immunodeficiency **virus** and cellular prion **protein** with the viral RNA.  
AUTHOR: Moscardini Mila; Pistello Mauro; Bendinelli M; Ficheux Damien; Miller Jennifer T; Gabus Caroline; Le Grice Stuart F J; Surewicz Witold K; Darlix Jean-Luc  
CORPORATE SOURCE: Department of Biomedicine, University of Pisa, I-56127 Pisa, Italy.  
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (2002 Apr 19) 318 (1) 149-59.  
Journal code: 2985088R. ISSN: 0022-2836.  
PUB. COUNTRY: England: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals  
ENTRY MONTH: 200207  
ENTRY DATE: Entered STN: 20020611  
Last Updated on STN: 20020713  
Entered Medline: 20020712

AB All lentiviruses and oncoretroviruses examined so far encode a major nucleic-acid binding protein (nucleocapsid or NC\* protein), approximately 2500 molecules of which coat the dimeric RNA genome. Studies on HIV-1 and MoMuLV using in **vitro** model systems and in vivo have shown that NC protein is required to chaperone viral RNA dimerization and packaging during virus assembly, and proviral DNA synthesis by reverse transcriptase (RT) during infection. The human cellular prion protein (PrP), thought to be the major component of the agent causing transmissible spongiform encephalopathies (TSE), was recently found to possess a strong affinity for nucleic acids and to exhibit chaperone properties very similar to HIV-1 NC protein in the HIV-1 context in **vitro**. Tight binding of PrP to nucleic acids is proposed to participate directly in the prion disease process. To extend our understanding of lentiviruses and of the unexpected nucleic acid chaperone properties of the human prion protein, we set up an in **vitro** system to investigate replication of the feline immunodeficiency virus (FIV), which is functionally and phylogenetically distant from HIV-1. The results show that in the FIV model system, NC protein chaperones viral RNA dimerization, primer **tRNA**(Lys,3) annealing to the genomic primer-binding site (PBS) and minus strand DNA synthesis by the homologous FIV RT. FIV NC protein is able to trigger specific viral DNA synthesis by inhibiting self-priming of reverse transcription. The human prion protein was found to mimic the properties of FIV NC with respect to primer **tRNA** annealing to the viral RNA and chaperoning minus strand DNA synthesis.  
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L9 ANSWER 3 OF 44 MEDLINE DUPLICATE 2  
ACCESSION NUMBER: 2000270264 MEDLINE  
DOCUMENT NUMBER: 20270264 PubMed ID: 10809779  
TITLE: In **vitro** studies on **tRNA** annealing and reverse transcription with mutant HIV-1 RNA templates.  
AUTHOR: Beerens N; Berkhout B  
CORPORATE SOURCE: Department of Human Retrovirology, Academic Medical Center, University of Amsterdam, Amsterdam 1100 DE,

Searcher : Shears 308-4994

09/713687

SOURCE: The Netherlands.  
JOURNAL OF BIOLOGICAL CHEMISTRY, (2000 May 19) 275  
(20) 15474-81.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 200006  
ENTRY DATE: Entered STN: 20000629  
Last Updated on STN: 20000629  
Entered Medline: 20000621

AB The human immunodeficiency virus type 1 (HIV-1) RNA genome encodes a semistable stem-loop structure, the U5-PBS hairpin, which occludes part of the **tRNA** primer binding site (PBS). In previous studies, we demonstrated that mutations that alter the stability of the U5-PBS hairpin inhibit virus replication. A reverse transcription defect was measured in assays with the virion-extracted RNA-**tRNA** complexes. We now extend these studies with in **vitro** synthesized wild-type and mutant RNA templates that were tested in primer annealing and reverse transcription assays. The effect of annealing temperature and the presence of the **viral nucleocapsid protein** on reverse transcription was analyzed for the templates with a stabilized or destabilized U5-PBS hairpin, and in reactions initiated by **tRNA** or DNA primers. The results of this in **vitro** assay are consistent with the in vivo findings, in that both **tRNA** annealing and initiation of reverse transcription are sensitive to stable template RNA structure. Reverse transcription initiated by a DNA primer is less hindered by secondary structure in the RNA template than **tRNA** primed reactions. The inhibitory effect of template structure on **tRNA**-primed reverse transcription is more pronounced in this in **vitro** assay compared with the in vivo material, indicating that the heat-annealed RNA-**tRNA** complex differs from the virion-extracted viral RNA-**tRNA** complex.

L9 ANSWER 4 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
ACCESSION NUMBER: 2000:703994 SCISEARCH  
THE GENUINE ARTICLE: 352XH  
TITLE: Zinc finger structures in the human immunodeficiency virus type 1 **nucleocapsid protein** facilitate efficient minus- and plus-strand transfer  
AUTHOR: Guo J H; Wu T Y; Anderson J; Kane B F; Johnson D G; Gorelick R J; Henderson L E; Levin J G (Reprint)  
CORPORATE SOURCE: NICHD, MOL GENET LAB, NIH, BLDG 6B, RM 216, BETHESDA, MD 20892 (Reprint); NICHD, MOL GENET LAB, NIH, BETHESDA, MD 20892; NCI, AIDS VACCINE PROGRAM, SAIC, FREDERICK CANC RES & DEV CTR, FREDERICK, MD 21702  
COUNTRY OF AUTHOR: USA  
SOURCE: JOURNAL OF VIROLOGY, (OCT 2000) Vol. 74, No. 19, pp. 8980-8988.  
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.  
ISSN: 0022-538X.

Searcher : Shears 308-4994

09/713687

DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 83

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The **nucleocapsid protein** (NC) of human immunodeficiency **virus** type 1 (HIV-1) has two zinc fingers, each containing the invariant metal ion binding residues CCHC. Recent reports indicate that mutations in the CCHC motifs are deleterious for reverse transcription in vivo. To identify reverse transcriptase (RT) reactions affected by such changes, we have probed zinc finger functions in NC-dependent RT-catalyzed HIV-1 minus- and plus-strand transfer model systems. Our approach was to examine the activities of wild-type NC and a mutant in which all six cysteine residues were replaced by serine (SSHS NC); this mutation severely disrupts zinc coordination. We find that the zinc fingers contribute to the role of NC in complete **tRNA** primer removal from minus-strand DNA during plus-strand transfer. Annealing of the primer binding site sequences in plus-strand strong-stop DNA [(+) SSDNA] to its complement in minus-strand acceptor DNA is not dependent on NC zinc fingers. In contrast, the rate of annealing of the complementary R regions in (-) SSDNA, and 3' viral RNA during minus-strand transfer is approximately eightfold lower when SSHS NC is used in place of wild-type NC. Moreover, unlike wild-type NC, SSHS NC has only a small stimulatory effect on minus-strand transfer and is essentially unable to block TAR-induced self-priming from (-) SSDNA. Our results strongly suggest that NC zinc finger structures are needed to unfold highly structured RNA and DNA strand transfer intermediates. Thus, it appears that in these cases, zinc finger interactions are important components of NC nucleic acid chaperone activity.

L9 ANSWER 5 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
ACCESSION NUMBER: 2000:703972 SCISEARCH  
THE GENUINE ARTICLE: 352XH  
TITLE: Human immunodeficiency **virus** type 1 **nucleocapsid protein** can prevent self-priming of minus-strand strong stop DNA by promoting the annealing of short oligonucleotides to hairpin sequences  
AUTHOR: Driscoll M D; Hughes S H (Reprint)  
CORPORATE SOURCE: NCI, HIV DRUG RESISTANCE PROGRAM, FREDERICK CANC RES & DEV CTR, POB B, FREDERICK, MD 21702 (Reprint); NCI, ABL BASIC RES PROGRAM, FREDERICK CANC RES & DEV CTR, FREDERICK, MD 21702  
COUNTRY OF AUTHOR: USA  
SOURCE: JOURNAL OF VIROLOGY, (OCT 2000) Vol. 74, No. 19, pp. 8785-8792.  
Publisher: AMER SOC MICROBIOLOGY, 1752 N ST NW, WASHINGTON, DC 20036-2904.  
ISSN: 0022-538X.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 41

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Understanding how viral components collaborate to convert the human immunodeficiency virus type 1 genome from single-stranded RNA

into double-stranded DNA is critical to the understanding of viral replication. Not only must the correct reactions be carried out, but unwanted side reactions must be avoided. After minus-strand strong stop DNA (-ssssDNA) synthesis, degradation of the RNA template by the RNase H domain of reverse transcriptase (RT) produces single-stranded DNA that has the potential to self-prime at the imperfectly base paired TAR hairpin, making continued DNA synthesis impossible. Although nucleocapsid protein (NC) interferes with -ssssDNA self-priming in reverse transcription reactions in *vitro*, NC alone did not prevent self-priming of a synthetic -ssssDNA oligomer. NC did not influence DNA bending and therefore cannot inhibit self-priming at hairpins by directly blocking hairpin formation. Using DNA oligomers as a model for genomic RNA fragments, we found that a 17-base DNA fragment annealed to the 3' end of the -ssssDNA prevented self priming in the presence of NC. This implies that to avoid self-priming, an RNA-DNA hybrid that is more thermodynamically stable than the hairpin must remain within the hairpin region. This suggests that NC prevents self-priming by generating or stabilizing a thermodynamically favored RNA-DNA heteroduplex instead of the kinetically favored TAR hairpin. In support of this idea, sequence changes that increased base pairing in the DNA TAR hairpin resulted in an increase in self-priming in *vitro*. We present a model describing the role of NC-dependent inhibition of self-priming in first-strand transfer.

L9 ANSWER 6 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
 ACCESSION NUMBER: 2000:265549 SCISEARCH  
 THE GENUINE ARTICLE: 299TW  
 TITLE: Interactions between human immunodeficiency virus type 1 reverse transcriptase, tRNA primer, and nucleocapsid protein during reverse transcription  
 AUTHOR: Hsu M; Wainberg M A (Reprint)  
 CORPORATE SOURCE: MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, 3755 CH COTE STE CATHERINE, MONTREAL, PQ H3T 1E2, CANADA (Reprint); MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, MONTREAL, PQ H3T 1E2, CANADA; MCGILL UNIV, DEPT MICROBIOL & IMMUNOL, MONTREAL, PQ H3T 1E2, CANADA  
 COUNTRY OF AUTHOR: CANADA  
 SOURCE: JOURNAL OF HUMAN VIROLOGY, (JAN-FEB 2000) Vol. 3, No. 1, pp. 16-26.  
 Publisher: LIPPINCOTT WILLIAMS & WILKINS, 530 WALNUT ST, PHILADELPHIA, PA 19106-3621.  
 ISSN: 1090-9508.  
 DOCUMENT TYPE: General Review; Journal  
 FILE SEGMENT: LIFE  
 LANGUAGE: English  
 REFERENCE COUNT: 108

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB An early step in the life cycle of human immunodeficiency virus type 1 is the reverse transcription of the viral RNA genome into double-stranded DNA, which is subsequently translocated to the cell nucleus. It is then integrated into host DNA and serves as a template for viral gene expression. Reverse transcription is catalyzed by the viral enzyme reverse transcriptase and is a complex process comprising a series of RNA-dependent DNA polymerization, DNA-dependent DNA polymerization, and RNase H reactions. Strand transfer reactions are required to complete the process. Re-verse

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transcription is initiated when a molecule of host cell **tRNA** (lys3). which serves as a primer, is bound to the primer binding site of **viral** genomic RNA. The **viral nucleocapsid protein** is involved in each of the initiation of reverse transcription and in subsequent strand transfer or template-switching events. We review the interactions among reverse transcriptase, viral genomic RNA, the **tRNA** primer of reverse transcription, and **viral nucleocapsid protein** in the various steps of reverse transcription, including primer placement, initiation, and processive synthesis.

L9 ANSWER 7 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)  
ACCESSION NUMBER: 1999:307393 SCISEARCH  
THE GENUINE ARTICLE: 186VV  
TITLE: Evidence of interactions between the nucleocapsid protein NCp7 and the reverse transcriptase of HIV-1  
AUTHOR: Druillennec S; Caneparo A; deRocquigny H; Roques B P (Reprint)  
CORPORATE SOURCE: UFR SCI PHARMACEUT & BIOL 4, DEPT PHARMACOCHEM MOL & STRUCT, CNRS, UMR 8600, INSERM, U266, F-75270 PARIS 06, FRANCE (Reprint); UFR SCI PHARMACEUT & BIOL 4, DEPT PHARMACOCHEM MOL & STRUCT, CNRS, UMR 8600, INSERM, U266, F-75270 PARIS 06, FRANCE  
COUNTRY OF AUTHOR: FRANCE  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (16 APR 1999) Vol. 274, No. 16, pp. 11283-11288.  
Publisher: AMER SOC BIOCHEMISTRY MOLECULAR BIOLOGY INC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814.  
ISSN: 0021-9258.  
DOCUMENT TYPE: Article; Journal  
FILE SEGMENT: LIFE  
LANGUAGE: English  
REFERENCE COUNT: 43

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The human immunodeficiency **virus** (HIV-1) **nucleocapsid protein** NCp7 containing two CX2CX4HX4C-type zinc fingers was proposed to be involved in reverse transcriptase (RT)-catalyzed proviral DNA synthesis through promotion of **tRNA**(3)(LYS) annealing to the RNA primer binding site, improvement of DNA strand transfers, and enhancement of RT processivity. The NCp7 structural characteristics are crucial because mutations altering the finger domain conformation led to noninfectious viruses characterized by defects in provirus integration. These findings prompted us to study a putative RT/NCp7 protein-protein interaction. Binding as says using far Western analysis or RT immobilized on beads clearly showed the formation of a complex between NCp7 and RT. The affinity of NCp7 for p66/p51RT was 0.60  $\mu$  M with a 1:1 stoichiometry. This interaction was confirmed by chemical cross-linking and co-immunoprecipitation of the two proteins in a viral environment. Competition experiments using different NCp7 mutants showed that alteration of the finger structure disrupted RT recognition giving insights into the loss of infectivity of corresponding HIV-1 mutants. Together with structural data on RT, these results suggest that the role of NCp7 could be to enhance RT processivity through stabilization of a p51-induced active form of the p66 subunit and open the way for designing new antiviral agents.



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L9 ANSWER 8 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
ACCESSION NUMBER: 1999:263770 BIOSIS  
DOCUMENT NUMBER: PREV199900263770  
TITLE: Papillomavirus capsid protein expression level  
depends on the match between codon usage and  
**tRNA** availability.  
AUTHOR(S): Zhou, Jian; Liu, Wen Jun; Peng, Shi Wen; Sun, Xiao  
Yi; Frazer, Ian (1)  
CORPORATE SOURCE: (1) Centre for Immunology and Cancer Research,  
University of Queensland, Princess Alexandra  
Hospital, Brisbane, QLD, 4102 Australia  
SOURCE: Journal of Virology, (June, 1999) Vol. 73, No. 6, pp.  
4972-4982.  
ISSN: 0022-538X.  
DOCUMENT TYPE: Article  
LANGUAGE: English  
SUMMARY LANGUAGE: English

AB Translation of mRNA encoding the L1 and L2 capsid proteins of  
papillomavirus (PV) is restricted in vivo to differentiated  
epithelial cells, although transcription of the L1 and L2 late genes  
occurs more widely. The codon composition of PV late genes is quite  
different from that of most mammalian genes. To test the possibility  
that PV late gene codon composition determines the efficiency of PV  
late gene expression in some cell types, synthetic bovine  
papillomavirus type 1 (BPV1) late genes were constructed with codon  
composition modified to resemble the typical mammalian gene.  
Expression of these genes from a strong promoter in Cos-1 cells was  
compared with expression of wild-type BPV1 late genes from the same  
promoter. Both unmodified and modified PV late genes were  
transcribed in Cos-1 cells, but only the codon-modified genes were  
translated. In **vitro** translation of wild-type but not  
synthetic BPV1 L1 mRNA was markedly enhanced by addition of  
aminoacyl-**tRNAs**. Codon composition thus limits BPV1 late  
gene translation in Cos-1 cells, and this limitation can be overcome  
by modification of the codon composition of the genes or by  
provision of excess **tRNA**. Replacement of codons in the  
green fluorescent protein (gfp) gene with those frequently used in  
PV late genes did not alter gfp transcription in Cos-1 cells but  
almost abolished translation, supporting the hypothesis that the  
observed differences in efficiency of translation of modified and  
unmodified PV capsid genes were related to codon usage rather than  
mRNA structure. As **tRNA** populations vary within and  
between tissues in the same eukaryotic organism, we speculate that  
matching of **tRNA** availability to codon usage may be one  
determinant of the restriction of expression of PV late genes to  
differentiated epithelium.

L9 ANSWER 9 OF 44 MEDLINE DUPLICATE 3  
ACCESSION NUMBER: 1999134376 MEDLINE  
DOCUMENT NUMBER: 99134376 PubMed ID: 9933645  
TITLE: Role of post-transcriptional modifications of primer  
tRNA<sup>Lys,3</sup> in the fidelity and efficacy of plus strand  
DNA transfer during HIV-1 reverse transcription.  
AUTHOR: Auxilien S; Keith G; Le Grice S F; Darlix J L  
CORPORATE SOURCE: LaboRetro ENS, INSERM U412, 46 allée d'Italie, 69364  
Lyon cedex 07, France.  
SOURCE: JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Feb 12) 274

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(7) 4412-20.  
Journal code: 2985121R. ISSN: 0021-9258.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199903  
ENTRY DATE: Entered STN: 19990324  
Last Updated on STN: 19990324  
Entered Medline: 19990311

AB During HIV reverse transcription, (+) strand DNA synthesis is primed by an RNase H-resistant sequence, the polypurine tract, and continues as far as a 18-nt double-stranded RNA region corresponding to the 3' end of tRNA<sup>Lys</sup>,3 hybridized to the viral primer binding site (PBS). Before (+) strand DNA transfer, reverse transcriptase (RT) needs to unwind the double-stranded **tRNA**-PBS RNA in order to reverse-transcribe the 3' end of primer tRNA<sup>Lys</sup>,3. Since the detailed mechanism of (+) strand DNA transfer remains incompletely understood, we developed an *in vitro* system to closely examine this mechanism, composed of HIV 5' RNA, natural modified tRNA<sup>Lys</sup>,3, synthetic unmodified tRNA<sup>Lys</sup>,3 or oligonucleotides (RNA or DNA) complementary to the PBS, as well as the **viral proteins** RT and **nucleocapsid protein** (NCp7). Prior to (+) strand DNA transfer, RT stalls at the double-stranded **tRNA**-PBS RNA complex and is able to reverse-transcribe modified nucleosides of natural tRNA<sup>Lys</sup>,3. Modified nucleoside m1A-58 of natural tRNA<sup>Lys</sup>,3 is only partially effective as a stop signal, as RT can transcribe as far as the hyper-modified adenosine (ms2t6A-37) in the anticodon loop. m1A-58 is almost always transcribed into A, whereas other modified nucleosides are transcribed correctly, except for m7G-46, which is sometimes transcribed into T. In contrast, synthetic tRNA<sup>Lys</sup>,3, an RNA PBS primer, and a DNA PBS primer are completely reverse-transcribed. In the presence of an acceptor template, (+) strand DNA transfer is efficient only with templates containing natural tRNA<sup>Lys</sup>,3 or the RNA PBS primer. Sequence analysis of transfer products revealed frequent errors at the transfer site with synthetic tRNA<sup>Lys</sup>,3, not observed with natural tRNA<sup>Lys</sup>,3. Thus, modified nucleoside m1A-58, present in all retroviral **tRNA** primers, appears to be important for both efficacy and fidelity of (+) strand DNA transfer. We show that other factors such as the nature of the (-) PBS of the acceptor template and the RNase H activity of RT also influence the efficacy of (+) strand DNA transfer.

L9 ANSWER 10 OF 44 MEDLINE DUPLICATE 4  
ACCESSION NUMBER: 1999214364 MEDLINE  
DOCUMENT NUMBER: 99214364 PubMed ID: 10196321  
TITLE: The human immunodeficiency virus type 1 Gag polyprotein has nucleic acid chaperone activity: possible role in dimerization of genomic RNA and placement of **tRNA** on the primer binding site.  
AUTHOR: Feng Y X; Campbell S; Harvin D; Ehresmann B; Ehresmann C; Rein A  
CORPORATE SOURCE: Retroviral Genetics Section, ABL-Basic Research Program, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Maryland

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21702, USA.  
SOURCE: JOURNAL OF VIROLOGY, (1999 May) 73 (5) 4251-6.  
Journal code: 0113724. ISSN: 0022-538X.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199905  
ENTRY DATE: Entered STN: 19990601  
Last Updated on STN: 19990601  
Entered Medline: 19990519

AB The formation of an infectious retrovirus particle requires several RNA-RNA interaction events. In particular, the genomic RNA molecules form a dimeric structure, and a cellular **tRNA** molecule is annealed to an 18-base complementary region (the primer binding site, or PBS) on the genomic RNA, where it will serve as primer for reverse transcription. **tRNAs** normally possess a highly stable secondary and tertiary structure; it seems unlikely that annealing of a **tRNA** molecule to the PBS, which involves unwinding of this structure, could occur efficiently at physiological temperatures without the assistance of a cofactor. Many prior studies have shown that the **viral nucleocapsid (NC) protein** can act as a nucleic acid chaperone (i.e., facilitate annealing events between nucleic acids), and the assays used to demonstrate this activity include its ability to catalyze dimerization of transcripts representing retroviral genomes and the annealing of **tRNA** to the PBS in **vitro**. However, mature NC is not required for these events in vivo, since protease-deficient viral mutants, in which NC is not cleaved from the parental Gag polyprotein, are known to contain dimeric RNAs with **tRNA** annealed to the PBS. In the present experiments, we have tested recombinant human immunodeficiency virus type 1 Gag polyprotein for nucleic acid chaperone activity. The protein was positive by all of our assays, including the ability to stimulate dimerization and to anneal **tRNA** to the PBS in **vitro**. In quantitative experiments, its activity was approximately equivalent on a molar basis to that of NC. Based on these results, we suggest that the Gag polyprotein (presumably by its NC domain) catalyzes the annealing of **tRNA** to the PBS during (or before) retrovirus assembly in vivo.

L9 ANSWER 11 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1999:205082 SCISEARCH

THE GENUINE ARTICLE: 173GG

TITLE: Time-resolved fluorescence investigation of the human immunodeficiency **virus** type 1 **nucleocapsid protein**: Influence of the binding of nucleic acids

AUTHOR: Bombarda E; Ababou A; Vuilleumier C; Gerard D; Roques B P; Piemont E; Mely Y (Reprint)

CORPORATE SOURCE: UNIV STRASBOURG 1, FAC PHARM, BIOPHYS LAB, CNRS, URA 491, BP 24, F-67401 ILLKIRCH GRAFFENS, FRANCE (Reprint); UNIV STRASBOURG 1, FAC PHARM, BIOPHYS LAB, CNRS, URA 491, F-67401 ILLKIRCH GRAFFENS, FRANCE; FAC PHARM, CNRS, URA D1500, INSERM, U266, UNITE PHARMACOCHEM MOL, F-75270 PARIS 06, FRANCE

COUNTRY OF AUTHOR: FRANCE

SOURCE: BIOPHYSICAL JOURNAL, (MAR 1999) Vol. 76, No. 3, pp.

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1561-1570.

Publisher: BIOPHYSICAL SOCIETY, 9650 ROCKVILLE PIKE,  
BETHESDA, MD 20814-3998.

ISSN: 0006-3495.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 54

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Depending on the HIV-1 isolate, MN or BH10, the nucleocapsid protein, NCp7, corresponds to a 55- or 71-amino acid length product, respectively. The MN NCp7 contains a single Trp residue at position 37 in the distal zinc finger motif, and the BH10 NCp7 contains an additional Trp, at position 61 in the C-terminal chain. The time-resolved intensity decay parameters of the zinc-saturated BH10 NCp7 were determined and compared to those of single-Trp-containing derivatives. The fluorescence decay of BH10 NCp7 could be clearly represented as a linear combination (with respect to both lifetimes and fractional intensities) of the individual emitting Trp residues. This suggested the absence of interactions between the two Trp residues, a feature that was confirmed by molecular modeling and fluorescence energy transfer studies. In the presence of *tRNA*(Phe), taken as a RNA model, the same conclusions hold true despite the large fluorescence decrease induced by the binding of *tRNA*(Phe). Indeed, the fluorescence of Trp(37) appears almost fully quenched, in keeping with a stacking of this residue with the bases of *tRNA*(Phe). Despite the multiple binding sites in *tRNA*(Phe), the large prevalence of ultrashort lifetimes, associated with the stacking of Trp(37), suggests that this stacking constitutes a major feature in the binding process of NCp7 to nucleic acids. In contrast, Trp(61) only stacked to a small extent with *tRNA*Phe. The behavior of this residue in the *tRNA*(Phe)-NCp7 complexes appeared to be rather heterogeneous, suggesting that it does not constitute a major determinant in the binding process. Finally, our data suggested that the binding of NCp7 proteins from the two HIV-1 strains to nonspecific nucleic acid sequences was largely similar.

L9 ANSWER 12 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.

ACCESSION NUMBER: 1999:87029 BIOSIS

DOCUMENT NUMBER: PREV199900087029

TITLE: Translation initiation at the CUU codon is mediated by the internal ribosome entry site of an insect picorna-like virus in *vitro*.

AUTHOR(S): Sasaki, Jun; Nakashima, Nobuhiko (1)

CORPORATE SOURCE: (1) Natl. Inst. Sericultural Entomol. Sci., Owashi, Tsukuba, Ibaraki 305-8634 Japan

SOURCE: Journal of Virology, (Feb., 1999) Vol. 73, No. 2, pp. 1219-1226.

ISSN: 0022-538X.

DOCUMENT TYPE: Article

LANGUAGE: English

AB AUG-unrelated translation initiation was found in an insect picorna-like virus, *Plautia stali* intestine virus (PSIV). The positive-strand RNA genome of the virus contains two nonoverlapping open reading frames (ORFs). The capsid protein gene is located in the 3'-proximal ORF and lacks an AUG initiation codon. We examined the translation mechanism and the initiation codon of the capsid

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protein gene by using various dicistronic and monocistronic RNAs in **vitro**. The capsid protein gene was translated cap independently in the presence of the upstream cistron, indicating that the gene is translated by internal ribosome entry. Deletion analysis showed that the internal ribosome entry site (IRES) consisted of approximately 250 bases and that its 3' boundary extended slightly into the capsid-coding region. The initiation codon for the IRES-mediated translation was identified as the CUU codon, which is located just upstream of the 5' terminus of the capsid-coding region by site-directed mutagenesis. In **vitro** translation assays of monocistronic RNAs lacking the 5' part of the IRES showed that this CUU codon was not recognized by scanning ribosomes. This suggests that the PSIV IRES can effectively direct translation initiation without stable codon-anticodon pairing between the initiation codon and the initiator methionyl-tRNA.

L9 ANSWER 13 OF 44 MEDLINE DUPLICATE 5  
ACCESSION NUMBER: 1999175320 MEDLINE  
DOCUMENT NUMBER: 99175320 PubMed ID: 10074407  
TITLE: Binding properties of the human immunodeficiency  
virus type 1 nucleocapsid  
protein p7 to a model RNA: elucidation of the  
structural determinants for function.  
AUTHOR: Urbaneja M A; Kane B P; Johnson D G; Gorelick R J;  
Henderson L E; Casas-Finet J R  
CORPORATE SOURCE: AIDS Vaccine Program, SAIC Frederick, National Cancer  
Institute-Frederick Cancer Research and Development  
Center, Frederick, MD, 21702-1201, USA..  
urbaneja@avpaxpl.ncifcrf.gov  
CONTRACT NUMBER: N01-CO-56000 (NCI)  
SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (1999 Mar 19) 287 (1)  
59-75.  
Journal code: 2985088R. ISSN: 0022-2836.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals; AIDS  
ENTRY MONTH: 199904  
ENTRY DATE: Entered STN: 19990426  
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Entered Medline: 19990415

AB HIV-1 nucleocapsid protein (NCp7) is a double zinc-fingered protein that has been traditionally implicated in viral RNA recognition and packaging, in addition to its tight association with genomic RNA and tRNA primer within the virion nucleocapsid. The availability of large quantities of viral or recombinant wild-type NCp7 and mutant p7 has made possible the assignment of the different roles that structural motifs within the protein play during RNA binding. At low ionic strength binding to the homopolymeric fluorescent poly(epsilonA), is electrostatically driven and four sodium ions are displaced. Arg7 in the flanking N-terminal region, Lys20 in the first zinc finger and one positively charged residue (attributed to Lys41) in the second zinc finger are involved in electrostatic contacts with RNA. The p7 zinc fingers do not bind independently but concomitantly. The first zinc finger (both isolated or in the context of the full-length protein) has a more prominent electrostatic interaction than the second one. T

zinc finger dominates the non-electrostatic stabilization of the binding to RNA due to stacking of its Trp residue with nucleic acid bases. Mutations in the highly conserved retroviral Zn-coordinating residues (CCHC) to steroid hormone receptor (CCCC) or transcription factor (CCHH) metal cluster types do not affect RNA binding. In spite of the limited impact in RNA binding affinity in **vitro** or RNA packaging in vivo that such mutations or structural alterations impart, they impair or abolish virus infectivity. It is likely that such an effect stems from the involvement of NCp7 in crucial steps of the virus life cycle other than RNA binding.  
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L9 ANSWER 14 OF 44 MEDLINE DUPLICATE 6  
 ACCESSION NUMBER: 1998440607 MEDLINE  
 DOCUMENT NUMBER: 98440607 PubMed ID: 9765488  
 TITLE: Roles of the human immunodeficiency virus type 1 **nucleocapsid protein** in annealing and initiation versus elongation in reverse transcription of viral negative-strand strong-stop DNA.  
 AUTHOR: Rong L; Liang C; Hsu M; Kleiman L; Petitjean P; de Rocquigny H; Roques B P; Wainberg M A  
 CORPORATE SOURCE: McGill University AIDS Centre, Lady Davis Institute-Jewish General Hospital, Montreal, Quebec, Canada H3T 1E2.  
 SOURCE: JOURNAL OF VIROLOGY, (1998 Nov) 72 (11) 9353-8. Journal code: 0113724. ISSN: 0022-538X.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals; AIDS  
 ENTRY MONTH: 199811  
 ENTRY DATE: Entered STN: 19990106  
 Last Updated on STN: 19990106  
 Entered Medline: 19981105

AB To study the initiation of human immunodeficiency virus type 1 reverse transcription, we have used the **viral nucleocapsid protein** (NC7) to anneal tRNA<sup>3</sup>Lys primer onto viral genomic RNA and have then eliminated NC7 from this primer-template complex by digestion with proteinase K and phenol-chloroform extraction of residual protein. Our data show that saturating concentrations of NC7 resulted in the formation of an active **tRNA**-template complex that yielded enhanced production of full-length negative-strand strong-stop DNA [(-)ssDNA] and that this complex remained active even after the elimination of NC7. While both of the two Zn finger motifs found within NC7 were essential for efficient elongation, NC protein that contained a point mutation in the first Zn finger or that was devoid of both Zn fingers yielded primer-template complexes that could still be initiated in 1-base-extension assays. In contrast, the use of heat annealing to produce primer-template complexes resulted in proportions of full-length (-)ssDNA lower than those seen with NC protein, and the addition of NC protein to such preformed primer-template complexes was able to reverse this defect only to a marginal extent.

L9 ANSWER 15 OF 44 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
 ACCESSION NUMBER: 1998:428519 BIOSIS

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DOCUMENT NUMBER: PREV199800428519  
TITLE: The yeast Ty3 retrotransposon contains a 5'-3' bipartite primer-binding site and encodes nucleocapsid protein NCp9 functionally homologous to HIV-1 NCp7.  
AUTHOR(S): Gabus, Caroline; Ficheux, Damien; Rau, Michael; Keith, Gerard; Sandmeyer, Suzanne; Darlix, Jean-Luc (1)  
CORPORATE SOURCE: (1) LaboRetro, Unite Virologie Humaine, INSERM, Ecole Normale Supérieure Lyon, 46 Allée d'Italie, 69364 Lyon France  
SOURCE: EMBO (European Molecular Biology Organization) Journal, (Aug. 17, 1998) Vol. 17, No. 16, pp. 4873-4880.  
ISSN: 0261-4189.  
DOCUMENT TYPE: Article  
LANGUAGE: English

AB Retroviruses, including HIV-1 and the distantly related yeast retroelement Ty3, all encode a nucleoprotein required for virion structure and replication. During an *in vitro* comparison of HIV-1 and Ty3 nucleoprotein function in RNA dimerization and cDNA synthesis, we discovered a bipartite primer-binding site (PBS) for Ty3 composed of sequences located at opposite ends of the genome. Ty3 cDNA synthesis requires the 3' PBS for primer tRNA<sup>i</sup>Met annealing to the genomic RNA, and the 5' PBS, in cis or in trans, as the reverse transcription start site. Ty3 RNA alone is unable to dimerize, but formation of dimeric tRNA<sup>i</sup>Met bound to the PBS was found to direct dimerization of Ty3 RNA-tRNA<sup>i</sup>Met. Interestingly, HIV-1 nucleocapsid protein NCp7 and Ty3 NCp9 were interchangeable using HIV-1 and Ty3 RNA template-primer systems. Our findings impact on the understanding of non-canonical reverse transcription as well as on the use of Ty3 systems to screen for anti-NCp7 drugs.

L9 ANSWER 16 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 1998:322473 SCISEARCH

THE GENUINE ARTICLE: ZJ231

TITLE: Endogenous reverse transcriptase assays reveal synergy between combinations of the M184V and other drug resistance-conferring mutations in interactions with nucleoside analog triphosphates

AUTHOR: Quan Y D; Gu Z X; Li X G; Liang C; Parniak M A; Wainberg M A (Reprint)

CORPORATE SOURCE: MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, MONTREAL, PQ H3T 1E2, CANADA (Reprint); MCGILL UNIV, JEWISH GEN HOSP, AIDS CTR, MONTREAL, PQ H3T 1E2, CANADA; MCGILL UNIV, DEPT MED, MONTREAL, PQ, CANADA; MCGILL UNIV, DEPT MICROBIOL & IMMUNOL, MONTREAL, PQ, CANADA

COUNTRY OF AUTHOR: CANADA

SOURCE: JOURNAL OF MOLECULAR BIOLOGY, (27 MAR 1998) Vol. 277, No. 2, pp. 237-247.  
Publisher: ACADEMIC PRESS LTD, 24-28 OVAL RD, LONDON, ENGLAND NW1 7DX.  
ISSN: 0022-2836.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 56

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB Resistance of HIV-1 reverse transcriptase (RT) to nucleoside analogs (e.g. AZT, ddC and 3TC) is conferred by various amino acid substitutions or combinations thereof on the RT molecule. The M184V mutation, that confers high and low-level resistance to 3TC and ddC, respectively, can restore sensitivity to AZT when introduced into RT against a background of AZT-resistance. The K65R mutation, that confers low level resistance to both 3TC and ddC, can also restore sensitivity to AZT. This information is of potential utility in choosing combinations of anti-viral drugs for clinical use. To explore this subject further, we have used an endogenous RT reaction to study mutated viruses containing M184V alone or M184V combined with each of the K65R, E89G or both the M41L and T215Y substitutions. Endogenous assays possess the advantage of utilizing genomic RNA as template in a reaction mixture that includes each of **tRNA(Lys.3)** and **viral nucleocapsid protein**, necessary for specific initiation of reverse transcription, as well as all other viral proteins that might impact on this process. We now show that viruses containing both M184V and K65R displayed synergistic resistance to 3TC triphosphate (3TCTP), while the same combination yielded the same level of resistance to ddC triphosphate (ddCTP) as that manifested by K65R alone. The combination of M184V and E89G displayed synergistic resistance against ddCTP but not 3TCTP, while viruses containing only E89G were highly resistant to 3TCTP and displayed low-level resistance to ddCTP. The results show that endogenous RT assays can reveal variable synergistic, antagonistic, or neutral effects in regard to drug sensitivity, depending on the presence of specific amino acid substitutions in RT itself. (C) 1998 Academic Press Limited.

L9 ANSWER 17 OF 44 SCISEARCH COPYRIGHT 2002 ISI (R)

ACCESSION NUMBER: 97:181293 SCISEARCH

THE GENUINE ARTICLE: WK242

TITLE: Ordered aggregation of ribonucleic acids by the human immunodeficiency **virus** type 1 **nucleocapsid protein**

AUTHOR: Stoylov S P (Reprint); Vuilleumier C; Stoylova E; DeRocquigny H; Roques B P; Gerard D; Mely Y

CORPORATE SOURCE: BULGARIAN ACAD SCI, INST PHYS CHEM, BG-1040 SOFIA, BULGARIA (Reprint); UNIV STRASBOURG 1, FAC PHARM, CNRS, URA 491, LAB BIOPHYS, F-67401 ILLKIRCH GRAFFENS, FRANCE; UFR SCI PHARMACEUT & BIOL, CNRS URA 498, INSERM U266, DEPT CHIM ORGAN, F-75270 PARIS, FRANCE

COUNTRY OF AUTHOR: BULGARIA; FRANCE

SOURCE: BIOPOLYMERS, (MAR 1997) Vol. 41, No. 3, pp. 301-312. Publisher: JOHN WILEY & SONS INC, 605 THIRD AVE, NEW YORK, NY 10158-0012. ISSN: 0006-3525.

DOCUMENT TYPE: Article; Journal

FILE SEGMENT: LIFE

LANGUAGE: English

REFERENCE COUNT: 48

\*ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS\*

AB The nucleocapsid protein NCp7, which is the major genomic RNA binding protein of human immunodeficiency virus type 1, plays an important role in several key steps of the viral life cycle. Many of the NCp7 activities, notably the nucleic acid annealing and the genomic RNA wrapping ones, are thought to be linked to a nonspecific